PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *Ipomoea pes-caprae Linn.R.Br* (CONVOLVULACEAE)

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI-32

In partial fulfillment of the requirement

for the award of degree of

MASTER OF PHARMACY IN PHARMACOGNOSY

Submitted By

(Reg. No: 261220701)

DEPARTMENT OF PHARMACOGNOSY

COLLEGE OF PHARMACY

MADURAI MEDICAL COLLEGE

MADURAI – 625 020

APRIL – 2014

Prof. (Dr) A. ABDUL HASAN SATHALI, M.Pharm., Ph. D.,
PRINCIPAL (i/c),
College of Pharmacy,
Madurai Medical College,
Madurai- 625020.

CERTIFICATE

This is to certify that the dissertation entitled **"PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF THE LEAVES OF *Ipomoea pes-caprae Linn.R.Br.* (CONVOLVULACEAE)''** submitted by **Ms. P. ANITHA (Reg. No. 261220701)** in partial fulfilment of the requirement for the award of the degree of **MASTER OF PHARMACY** in **PHARMACOGNOSY** by The Tamil Nadu Dr. M.G.R. Medical University is a bonafied work done by him during the academic year 2013-2014 under my guidance at the Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai-625 020.

Date :

Station: **(Prof. Dr. A. ABDUL HASAN SATHALI M.Pharm., Ph.D.,)**

Mr T.VENKATARATHINAKUMAR, M.Pharm.,(Ph. D).,
Assistant Reader in pharmacy,
Department of pharmacognosy,
College of Pharmacy,
Madurai Medical College,
Madurai-625020

CERTIFICATE

This is to certify that the dissertation entitled **"PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF THE LEAVES OF *Ipomoea pes-caprae Linn.R.Br.* (CONVOLVULACEAE)''** submitted by **Ms. P. ANITHA (Reg. No. 261220701)** in partial fulfilment of the requirement for the award of the degree of **MASTER OF PHARMACY** in **PHARMACOGNOSY** by The Tamil Nadu Dr. M.G.R. Medical University is a bonafied work done by him during the academic year 2013-2014 under my guidance at the Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai-625 020.

Date :

Station : (**T. VENKATARATHINAKUMAR M.Pharm.,(Ph.D).,)**
Project
Supervisor

Dr. (Mr.) D. STEPHEN., M.Sc., Ph.D.,

ASSISTANT PROFESSOR,

DEPARTMENT OF BOTANY,

THE AMERICAN COLLEGE,

MADURAI - 625002.

CERTIFICATE

This is to certify that the specimen brought by **Miss. P. ANITHA ,** II.M. Pharmacy, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai is identified as ***Ipomoea pes-caprae*** **Linn.R.Br.** belonging to the family **Convolvulaceae**.

STATION : Madurai.

(Dr. D. STEPHEN)

DATE :

Dr. D. STEPHEN, Ph.D.,
LECTURER IN BOTANY
THE AMERICAN COLLEGE
MADURAI-625 002
TAMILNADU - INDIA

(For IAEC / CPCSEA usage)

Proposal number	: P.Anitha/M.Pharm/IAEC/122 /KMCP/ 261220701/2013-14
Date first received	:01.10.2013
Date received after modification (if any)	: NA
Date received after second modification (if any)	: NA
Approval date	: 10.10.2013
Expiry date	: 28.02.2014
Name of IAEC / CPCSEA chairperson	: N.CHIDAMBARANATHAN

Date:10.10.2013

Signature

10/10/13

CPCSEA NOMINEE
INSTITUTIONAL ANIMAL ETHICS COMMITTEE
K.M. COLLEGE OF PHARMACY
MADURAI-625 107

I. A. E. C. CHAIRMAN
INSTITUTIONAL ANIMAL ETHICAL COMMITTEE
K. M. COLLEGE OF PHARMACY
MADURAI-625 107.

ACKNOWLEDGEMENTS

I first and foremost express my revered regard and obeisance to the **ALMIGHTY GOD** with whose blessings I was able to complete my project work.

I am grateful to express my sincere thanks to **Dr. SANTHA KUMAR., M.SC (FSC), M.D(FM), PGDMLE, Dip.N.B (FM)** Dean, Madurai Medical College, Madurai for giving me an opportunity to carry out my project work.

I express my thanks to **Mrs. A.AJITHADAS ARUNA M.Pharm., Ph.D., Joint Director of Medical Education Pharmacy (JDM)**, Chennai.

I express my sincere thanks to **Prof. Dr. A. ABDUL HASAN SATHALI, M.Pharm., Ph.D,** Professor and Head of Department of Pharmaceutics, College of Pharmacy, Madurai Medical College, Madurai for their support, advice and valuable suggestions.

It is my privilege to express a deep and heartfelt sense of gratitude and my regards to our respected **Mr. T. VENKATARATHINAKUMAR, M.Pharm.,(Ph.D).,** Assistant reader in pharmacy, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College for his active guidance, advice, help, support and encouragement. I am very much obliged for his perseverance, without which my project work would not be completed.

I express my sincere thanks to **Dr. K. PERIYANAYAGAM, M. Pharm., Ph.D,** and **Miss. R. GOWRI M.Pharm.,** Assistant Readers in Pharmacy, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai for their support and valuable suggestions.

I thank **Prof. (Mrs) R. THARABAI, M.Pharm,** Professor and Head of Department of Pharmaceutical chemistry, Madurai Medical College, Madurai for their guidance during the course of my study.

I thank **Mrs. A. SETHURAMANI, M. Pharm.,** and **Dr.(Mrs). A. KRISHNAVENI, M. Pharm., Ph.D.,** Tutors in Pharmacy, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College for their help.

I bring forth my sincere thanks to **DR. Mrs. SOBANA, M. VSc.,** and **Mrs. PADMINI, M.Vsc.,** Veterinary Surgeon, Central Animal House, Madurai Medical College, Madurai, for their support in getting the ethical clearance and valuable suggestions regarding pharmacological study needed for this dissertation

I thank **Mr. P. SIVAKUMAR M.Sc.,** Lab Supervisor of the Dept. of Pharmacognosy and

I thank **Dr. A. MUTHURAMAN., M.Pharm., Ph.D.,** Department of Pharmacology, akal college of pharmacy, Punjab for grand me permission and **MR. S. SELVA KUMAR,** Department of Pharmacology, Madurai Medical College, Madurai for helping me in carrying out the anti-neuropathic activity.

I extend my thanks to all the teaching staff of College of Pharmacy and of Madurai Medical College who have rendered their help towards completion of the project.

I place on record my gratitude to **Dr. P. JAYARAMAN, M.Sc., Ph.D.,** Director, Plant Anatomy Research Centre, Chennai 600 045 who helped me in the microscopic studies and **Dr. D. STEPHEN, Ph.D.,** Senior Lecturer, Department of Botany, American College, Madurai who helped me in the identification of my plant.

I thank **Mr. Magudeeswaran DMLT**, and **Mrs. P. Ellayee, Mr. Tharumar,** for their help throughout my study.

I also thanks for my ever loving classmates **Miss. P . BALA, Ms. R. JANCY GRACELET, Ms. M. KALAIYARASI, Ms. S.R. NANTHINI BOSE, Ms. S. NATHIYA PRAKASH, Ms. D. SUGANYA, Ms. K. VIJAYA LAKSHMI, Mr. S.**

JEGADEESH and special thanks for **Mr. S.P. BOOPATHY, Mr. SANKAR GANESH, Mr. PRAVEEN KUMAR.**

I also thank my dear own brother **Mr. P. ARJUN KUMAR,** M.Pharm., Department of pharmaceutics, Madurai Medical college, Madurai.

I also thank my grateful friends **MS. S. KARPAGAM, MS. E. AJILA, R. ELAVARASI, MR. K. SASI KUMAR** M.Pharm., Department of pharmaceutical chemistry, Medical College, Madurai.

I also thanks for my PG Seniors and PG,UG Juniors special thanks for **J. RABISTON.**

Above all, I am forever indebted to my soul mother **Mrs. P. SIVAGAMI** and my sweetest sister **Ms. P. GOMATHY** and my dear brother in law **Mr. C. MARIAPPAN CARDS, Mr. S. SELVAM B.A., Mr. S. SURESH** Southern Railway Department, Tirunelveli for their understanding, endless patience, help and encouragement which made me to complete this work.

CONTENTS


Introduction

CHAPTER - 1

INTRODUCTION

HERBAL MEDICINE [1-3]

Ever since the birth of mankind there has been a relationship between disease and plants. There is no record that people in prehistoric times used synthetic medicines for their ailments. But they tried to make use of the things, which were easily available .i.e the plants and animals. They started using plants and found that majority of plants were suitable as food, where as other were either poisons or medicinally useful. By their experience this knowledge of herbal remedies was transferred to generation as folk medicine. So, the history of herbal medicine as old as human history.

Plants have been used for medicinal purpose long before and recorded in the history. For example ancient Chinese and Egyptian papyrus writing describe medicinal plants and their uses. Indigenous culture (eg: African and Native American) used traditional medicinal systems eg: ayurvedic and traditional Chinese medicine in which herbal therapies were used systematically. Scientists found that people in different parts of the globe tended to use the same or similar plants for the same purpose.

In the early 19^{th} century when method of chemical analysis first become available scientists started extracting and modifying the active ingredients from plants. Later chemists began making their own version of plant compounds, beginning the transition from raw herbs to synthetic pharmaceuticals. Over time, the use of herbal medicines declined in favour of pharmaceuticals.

Recently the WHO estimated that 80% of people worldwide rely on herbal medicine for some aspect of their primary healthcare. In the last twenty years in the United States, increasing public dissatisfaction with the cost of prescription medications combined with an interest in returning to natural or organic remedies has led to an increase in the use of herbal medicines. In Germany roughly 600 to 700 plant based medicines are available and are prescribed by approximately 70% of German physicians.

"The lord has created medicines out of the earth and he that is wise will not abhor them"

The WHO defines 4 types of herbal medicines:

- Raw herbs
- Herbal material –plant juice, gums, oils, resins, dry powders.
- Herbal preparations – extracts and tincture of herbal material produced by biological, chemical method such as extraction, fractionation, purification and concentration.-The basis for finished herbal products.
- Finished herbal products.

NEUROPATHY [4]

A classical term for any disorder affecting any segments of the nervous system.

NEUROPATHIC PAIN [5]

Neuropathy is a collection of disorders that occurs when nerves of the peripheral nervous system (the part of the nervous system outside of the brain and spinal cord) are damaged. The condition is generally referred to as **peripheral**

neuropathy and it is most commonly due to damage of nerve axons. Neuropathy usually causes pain and numbness in the hands and feet. It can result from traumatic injuries, infections, metabolic disorders and exposure to toxins.

Neuropathy can affect nerves that control muscle movement (motor nerves) and those that detect sensations such as coldness or pain (sensory nerves).In some cases of autonomic neuropathy-it can affect internal organs, such as the heart, blood vessels, bladder or intestines. Pain from peripheral neuropathy is often described as a tingling or burning sensation. There is no specific length of time that the pain exists, but symptoms often improve with time – especially if the neuropathy has an underlying condition that can be cured. The condition is often associated with poor nutrition. A number of diseases and pressure or trauma but many cases have no known reason and other wise called as idiopathic neuropathy.

CLASSIFICATION OF NEUROPATHY [6]

Peripheral neuropathy can be broadly classified into the following categories:

- Mononeuropathy – involvement of a single nerve. Examples include carpal tunnel syndrome,ulnar nerve palsy,radial nerve palsy and peroneal nerve palsy.
- Multiple mono neuropathy – two or more nerves individually affected.
- Polyneuropathy – generalized involvement of peripheral nerves. Examples include diabetic neuropathy and Guillain – Barre syndrome.

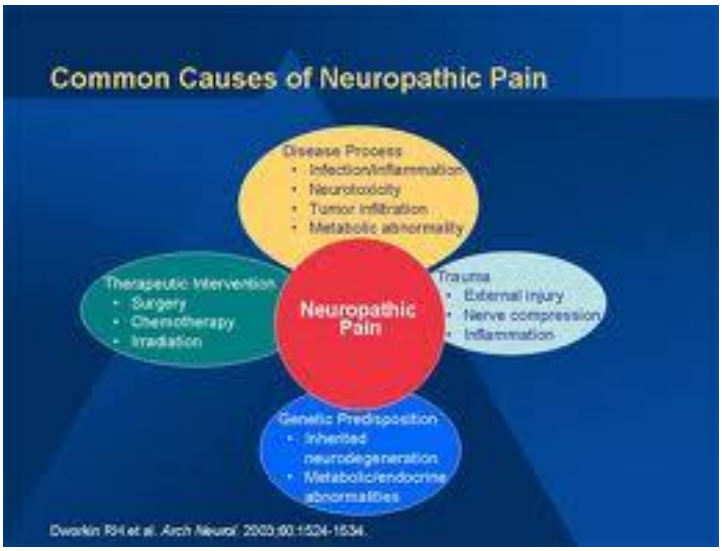
Neuropathy may also be categorized based on functional classification (motor,sensory,autonomic or mixed)or the type of onset (acute – hours or days, sub-acute – weeks or months or chronic – months or years). The most common form of neuropathy is (symmetrical) peripheral polyneuropathy, which mainly affect the feet and legs on both sides of the body.

CAUSES OF NEUROPATHY [7,8]

About 30% of neuropathy cases are considered idiopathic, which means they are of unknown cause. Another 30% of neuropathic are due to diabetic. In fact, about 50% of people with diabetic are susceptible to some type of neuropathy. The remaining cases of neuropathy are called as acquired neuropathies and these diseases have several possible causes including

- Trauma or pressure on nerves, often from repetitive motion such as typing on a keyboard.
- Nutritional problems and vitamins deficiencies, often from a lack of B vitamins.
- Alcoholism, often through poor dietary habits and vitamins deficiencies.
- Autoimmune diseases, such as lupus, rheumatoid arthritis and GulianBarre syndrome.
- Tumors, which often press up against nerves.
- Other disease and infections, as kidney disease,liver disease,lymph disease, HIV/AIDS or hypothyroidism.
- Inherited disorders (hereditary neuropathies)such as Charcot – Marie – Tooth disease and amyloid polyneuropathy.
- Poison exposure from toxins such as heavy metals and certain medications and drug induced neuropathic pain as a result of cancer treatment.

FIG 1 : Commom causes of neuropathic pain



ANIMAL MODELS OF NEUROPATHIC PAIN [9]

Chemical induced model

a, Acrylamide induced neuropathy.

Disease induced model

a, Diabetic induced model

b, cancer pain model

3.peripheral nerve injury model

a, Chronic constriction injury model

b, Complete sciatic nerve transition model

c, Partial sciatic nerve ligation model

d, Spinal nerve ligation model

e, Tibal and sural nerve transition

f, Common peroneal nerve ligation model

4 .Drug induced model

a, Vincristine induced model

b, Anti HIV drug induced model

5.Micellaneous model

a, Chronic ethanol consumption/withdrawal induced neuropathy

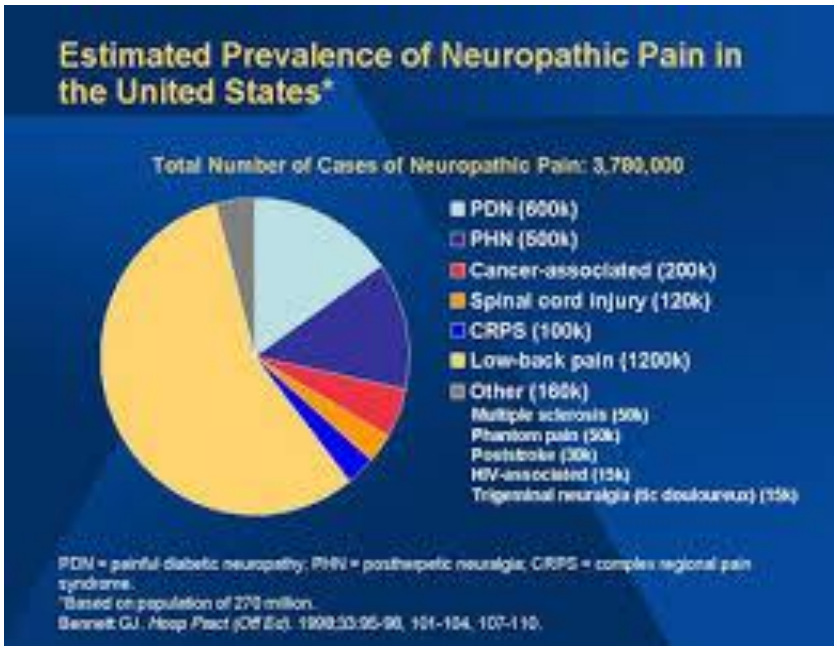
b ,Pyridoxine deficiency induced neuropathy

HERBS USED TO CURE NEUROPATHY [10]

Neuropathy is a term that refers to problems associated with the peripheral nervous system and it may cause muscle weakness,spasms,cramps and pain. These symptoms can be easily triggered by outside stimuli and they may occur over again unless treated. There are some herbal remedies that cure or treat these conditions without the sideeffects of allopathic medicine.

- *Hypericumper foratum.*
- *Polygonummultiflorum.*
- *Levisticum officinale.*
- *Filipendula ulmaria.*

FIG 2 : Estimated prevalence of neuropathic pain in USA



INFLAMMATION [11]

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens damaged the cells. The classical signs of inflammation are :

- Pain
- Heat
- Redness
- Swelling
- Loss of function

Inflammation can be classified as

1. Acute:

   Characterized by local vasodilatation and increased capillary permeability.

2. Sub- acute :

   Characterized by infiltration of leukocytes and phagocytic cells.

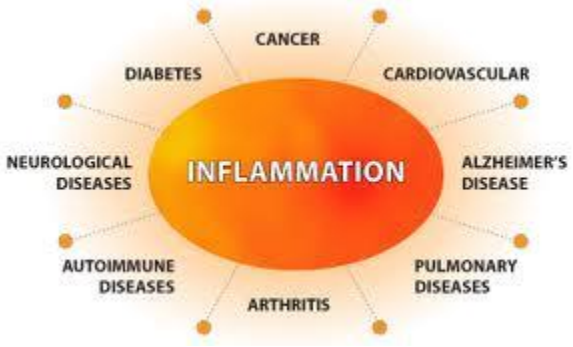
3. Chronic :

   Tissue degeneration and fibrosis.

CAUSES OF INFLAMMATION

The main reasons of inflammation are burns, ionizing radiation, toxins, stress, trauma, and alcohol.

FIG 3: Causes of inflammation



ANIMAL MODELS FOR INFLAMMATION [12]

I) Acute and sub-acute inflammation:

1, Paw edema

2, Croton oil ear edema

3, Pleurisy test

4, Ultra violet erythema

5, Oxazolone induced ear edema

6, Granuloma pouch technique

7, Vascular permeability

II) Proliferative phase:

- Cotton pellet granuloma
- Adjuvant arthritis
- Sponge implantation technique
- Glass rod granuloma

HERBS USED TO CURE INFLAMMATION[13]

- *Ocimum sanctum (holy basil).*
- *Curcuma longa (turmeric).*
- *Boswellia serrate (indianolibanum).*
- *Ginger.*

LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

ETHNO-MEDICINAL INFORMATION [14]

- The plant is extensively used as an anti- inflammatory.
- It is used in folk medicine.
- The plant extracts are used as medicine to treat fatigue and venereal disease.
- The leaf is used for skin infection, inflammation and laxative.
- The decoction of the roots and leaves are used in rheumatism, gout and gonorrhea.

PHYTOCHEMISTRY

Pareda mirinda *et al.*,(2005) have studied the hexane soluble extract of aerial parts of the drug *Ipomoea pes-caprae* (morning glory) by preparative scale HPLC and reported six lipophilic glycosides.[15]

Martinez *et al.*,(2008) have studied the hexane soluble extracts from the leaf of *Ipomoea pes-caprae* by preparative scale HPLC and reported the resin glycosides.[16]

Deepak venkataraman *et al*.,(2013) have studied the phytochemistry of ethanolic extracts of *Ipomoea pes-caprae*. The study revealed the presence of alkaloids, carbohydrate, flavonoid, tannin, glycosides, terpenoids and sterols. [17]

PHARMACOLOGY

Pongprayoon *et al.,* (1991) have studied the effect of extract of *Ipomoea pes-caprae* in the treatment of dermatitis caused by poisonous jelly Fish toxins. The extract also exhibited significant antispasmodic activity in isolated guinea pig ileum and anti-inflammatory activity in carrageen an induced in rat paw edema.[18]

Philippi *et al*.,(2010) have reported immunostimulatory activity from Calophyllum brasilliense, *Ipomoea pes-caprae* and *Mutayaba elaeagnoides* by human peripheral blood mononuclear cell proliferation. The result reveals that significant immunostimulatory activity of these plants. [19]

Deepak venkataraman *et al*.,(2013) have showed anti-inflammatory activity of ethanolic extract from aerial parts of *Ipomoea pes-caprae* using cotton pellet induced granuloma model.[20]

Umamaheshwari *et al.*,(2012) have studied the antioxidant and radical scavenging activity of ethanolic extract of *Ipomoea pes-caprae.*[21]

Deepak venktaraman *et al*.,(2013) have reported ethanolic extracts of *Ipomoea pes-caprae* was showed antiulcer effect(200 and 400 mg/kg) in aspirin and pyloric ligation induced gastric ulcer models in wistar rats. Ranitidine used as a standard.[22]

Ramanathan *et al.*,(2011) have reported the *in vitro* antifungal activity ethanolic extract of *Ipomoea pes-caprae* by agar diffusion method.[23]

OTHER SPECIES

PHARMACOGNOSY

Martins *et al.*,(2012) have studied the leaf anatomy and histochemistry for *Ipomoea asarifolia.* [24]

Rajendran *et al*.,(2008) have studied the pharmacognostical Identification of stem and root of *Ipomoea quamoclit.*[25]

Siddiqi *et al.*,(1990) have evaluated the botanical characters of *Ipomoea hederarea.*[26]

PHYTOCHEMISTRY

Ono *et al.*,(2010) have discussed the resin glycosides from the leaves and stem of *Ipomoea digitata.* The leaves and stem of the ipomoea gave six organic acids.[27]

Nair *et al.*,(2010) have reported the chemical and nutrient analysis of the weed *Ipomoea carne*..The study revealed the presence of flavonoids, tannins, alkaloids, amino acid, phenolicacid, sugar and saponins.[28]

Yin *et al.*,(2009) have reported the three new pentasaccharide resin glycosides from the root of *Ipomoea batatas.* [29]

Yahara *et al.*,(2002) have isolated the phenolic compounds from the methanolic extract of dried leaves of *Ipomoea batatas* and these compounds were identified by spectral studies.[30]

Singh Gupta *et al.*,(1995) have isolated a water soluble seed gum polysaccharide containing D-galactose and D-mannose as sugars from *Ipomoea pes-tigridis* and its structural elucidation has also been described. [31]

Ono *et al.*,(1991) have isolated the ether soluble resin Glycosides, operculins from the roots of *Ipomoea operculata.* They were characterized on the basis of chemical and spectral analysis.[32]

Wilkinson *et al.*,(1987) have isolated the seed alkaloids from *Ipomoea coccinea, Ipomoea wrightii* and these alkaloids were quantified by spectrophotometry as ergonovine maleate equivalents.[33]

Harrison *et al.*,(1986) have examined the four new fatty acid glycosides from *Ipomoea dichroa.* They were identified as dichrosides A, B, C and D besides friedelin, stearic acid, β-sitosterol and its glycosides. [34]

Schneider *et al.*,(1984) have isolated nine new sesquiterpenes from the root *Ipomoea batatas* and their structure were also determined.[35]

PHARMACOLOGY;

Haw *et al.*,(2012) have reported the hypouricemic effect of anthocyanin extracts of *Ipomoea batatas* on potassium oxolate induced hyperuricemic in mice [36].

Leon Rivera *et a*l.,(2011) have demonstrated the sedative, Vasorelaxant, cytotoxic effect of convolvulin from the root extract of *Ipomoea tyrianthina*[37].

Chimkode *et al.*,(2010) have reported the wound healing activity from the tuberous root extract of *Ipomoea batata in vivo* model [38].

Lin *et al.*,(2009) have studied the cytotoxic activity of the ethanolic extract from *Ipomoea cairica*[39].

Herrera-Ruiz *et al.*,(2008) have reported the central nervous system depressant activity of ethylacetate extract from *Ipomoea stans* root .The administration of this extract produced an anxiolytic effect in mice(20 -40 mg/kg) [40].

DattaChoudry *et al.*,(2007) have studied the chemical characterization and antifungal and CNS depressant activity of *Ipomoea aquatica.* The methanolic extract of *Ipomoea aquatic* showed the antifungal activity against *candida albicans*(1.6cm) and for CNS depressant activity in mice which was almost equal to the effect of standard drug[41].

Ferreira *et al.*,(2006) have evaluated anti nociceptive effect of *Ipomoea cairica.* The ethanolic extract (100,300,1000 and 3000 mg/kg; *p.o.*) of this plant induced dose dependent reduction of response in the formalin test inflammatory phase in mice model. The same dose range did not modify neurogenic pain in formalin test and tail flick reflex latency in carrageenan induced paw edema and rota –rod test[42].

Leon *et al.*,(2005) have estimated pentasaccharide glycosides from the root of *Ipomoea murucoides*. These constituents were evaluated for cytotoxicity against of cancer cell lines[43].

Yoshimoto *et al.*,(2004) have estimated the phenolic composition and radical scavenging activity of sweet potato *Ipomoea batatas* treated with koji [aspergillus awamori and cellulose (cellulosinT2) [44].

Haueza *et al.*,(2003) have reported the immunomodulatory activity of *Ipomoea carnea* on peritoneal cells of rats model. The experimental model suggested that low doses of *Ipomoea carnea* induced enhanced phagocytosis activity and hydrogen peroxide production by macrophages [45]

Mastui *et al.*,(2002) have performed anti-hyperglycemic effect of diacylated anthocyanin isolated from *Ipomoea batatas*. They suggested that anthocyanin possess alpha glucosidase inhibitory activity after a single oral administration in 8weeks old male rats[46].

Kusano *et al*.,(2001) have reported isolation of anti-diabetic components from *Ipomoea batatas*[47].

Navarro-Ruiz *et al.*,(1996) have demonstrated the anti-convulsant effect of aqueous hydro-alcoholic and chloroform extracts from roots of *Ipomoea stans*. Maximal electroshock seizure inducing test(MES) and subcutaneously injected metrazole (METSC)were the premental epilepsy model used and maximum activity was seen with aqueous extract[48].

Reynolds *et al.*,(1995) have isolated and characterized the cytotoxic and antibacterial tetrasaccharide glycoside from *Ipomoea stans.* These compounds have

pronounced cytotoxicity towards three human tumor cell lines as well as specific antibiotic activity against two bacterial strains [49].

Reza *et al.*,(1994) have studied the antibacterial activity of the chloroform extract of different parts of *Ipomoea fistulosa* against all the strains of *shigella* and the gram positive bacteria namely *Bacillus megaterium* and *Bacillus polymyxa*. The extract exhibited significant anti-bacterial activity.[50]

Dasilvo Filho *et al.*,(1986) have described the isolation of four new antimicrobial glycosides from *Ipomoea bahensis.* They showed significant activity against sarcoma 180 in mice model. [51]

AIM AND SCOPE

CHAPTER - 3

AIM AND SCOPE

Ipomoea pes-caprae.Linn.R.Br. **(**convolvulaceae) is a creeper and twinning, glabrous herb. The leaves often broad than long, fleshy, orbicular, obtuse emarginated and bilobed. The plant is very common and this abundant weed was distributed throughout India in the sandy shoves and sandy river banks. It also grows near the sea and other sandy areas throughout India. [52]

The ethno-medicinal information revealed that almost all parts of the plant are employed in various indigenous system of medicine to treat several diseases;

- *Ipomoea pes- caprae* is used for the treatment of rheumatoid arthritis.
- The leaves are used in stomachic and nervine tonic.
- The plant is used for insulinogenic and hypoglycemic activity as well as antinociceptive properties.
- *Ipomoea pes-caprae* is often used by fisherman to treat jelly fish sting.
- The roots have diuretic properties and used to treat bladder problems, stranguary, dysuria and oedema.

According to the literature survey, this plant has not been reported for painful neuropathy though ethno-medicinal information indicates that it has been used for the treatment of nervous disorders.

So, the present work has been designed to carry out the following works on the leaves of *Ipomoea pes-caprae.*

1. It was planned to carry out the detailed pharmacognostical studies on this leaves of this plant.

2. Preliminary phytochemical studies were carried out for the crude drug as well as extracts of this plant in order to confirm the presence of phytoconstituents like flavonoids, tannins, glycosides, carbohydrates and saponins.

3. To estimate the total phenolic, flavonoid and tannin content in the extracts of this plant

4. Phytochemical evaluation including identification and characterization of the active principles by HPLC.

5. It was planned to evaluate the extract of this plant for the following pharmacological studies.

A. Determination of antioxidant potential of extracts by,

- Hydrogen peroxide scavenging activity.
- DPPH (2,2,diphenyl-2-picryl hydrazyl) assay
- Phosphomolybdenum method
- Reducing power assay

B. Pharmacological evaluation of *Ipomoea pes-caprae* against vincristine induced neuropathic pain in rats.

C. *In vitro* anti-inflammatory activity of the plant extract by Human red blood corpuscles membrane stabilization model (HRBM).

PLANT PROFILE

CHAPTER – 4

PLANT PROFILE

Ipomoea pes-caprae Linn.R.Br. is a creeper and twinning, glabrous herb belonging to the family convolvulaceae.

SYNONYM[53]

Ipomoea biloba,beach morning glory,railroad vine.

SYSTEMATIC POSITION

Kingdom	-	planta
Subkingdom	-	tracheophyta
Super division	-	Spermatophyta
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Sub class	-	Asteridae
Order	-	Solanales
Family	-	Convolvulaceae
Genus	-	***Ipomoea***
Species	-	***pes-caprae***

VERNACULAR NAMES

Sanskrit	-	Vriddadaru
Hindi	-	Dopatilata
Bengali	-	chagalkhuri
Tamil	-	Adambuvalli,adappankodi
Malayalam	-	Chuvannaadambu
English	-	Goat’s foot creeper
Gujarathi	-	Ravarapatri
Telugu	-	Balabandatige

GEOGRAPHICAL DISTRIBUTION [54]

Ipomoea pes-caprae is a pan tropical vine. It is distributed South Atlantic, Gulf coasts, Texas, Mexico and throughout peninsular Florida. It is established worldwide on many tropical beaches including those of Australia and the Caribbean.

HABITAT OF PLANT

Rail road vine grows rapidly, but unevenly. Its common name can be attributed its ability to send out "tracks" of stolons more than 100 feet long. Stolons are similar to stems except they produce adventitious roots at the nodes and run horizontally rather than vertically. Taproots are deep, sometimes penetrating 3 feet into the soil. This ground-hugger usually grows to a height of more than 16 inches tall but, can form a dense groundcover as much as 30 feet across.

DESCRIPTION OF PLANT

LEAVES

A creeper and twinning glabrous herb. Leaves are broader than long. It is fleshy, orbicular, obtuse, emarginated or bilobed and prominently nerved.

STEM

The new stem is up to 0.5 inches in diameter.

FRUIT

Fruit is globes about 13-20 mm diameter. Calyx lobe is persistent at the base. Pedicels are about 50 mm long.

FLOWER

Auxiliary, funnel shaped flowers are pink to lavender purple colour. It is about 2 inches long. Corollas are 3-6 cm in length.

SEED

Seeds are black. It bears trigonous- globose shape.

PHARMACOGNOSTIC STUDIES

CHAPTER 5

MATERIALS AND METHODS

PART I – PHRAMACOGNOSTICAL STUDIES

SECTION- A

MACROSCOPY OF THE LEAVES [55]

Organoleptic characters such as colour, odour, texture, shape, size, apex, venation and arrangement of the leaves were studied for this plant.

The photographic representation of the macroscopic features are presented in **Fig 4 – 10.**

SECTION - B

MICROSCOPICAL STUDIES OF THE LEAVES [56]

Collection of plant material

Plants were collected from Ramnad and identified by taxonomist.

Specimen collection

Petiole and leaf were fixed in FAA solution (70% ethyl alcohol, formalin and acetic acid in the ratio of 90 mL: 5 mL: 5 mL). The materials were kept in the fluid for three days, after which they were washed in water and dehydrated with tertiary butyl alcohol. Paraffin wax was filtered and the specimens were embedded in wax for sectioning[57-61].

Sectioning

Transverse sections of petiole and leaf were taken using microtome and stained with toluidine blue. All sides, after staining in toluidine blue were dehydrated by employing graded series of ethyl alcohol (70 %, 90%, 100% alcohol) and xylol-alcohol (50-50) and passed through xylol and mounted in DPX mountant[62-64].

Clearing of leaves

Clearing of leaves for studying stomatal number and stomatal index was done by using 5% sodium hydroxide with chlorinated soda solution supplemented with gentle heat. Quantitative microscopy of leaves was carried out was studied as per the procedure given standard procedure. Photomicrographs were taken with the help of Nikon Eclipse E200 Microscope. Powder microscopy of the leaf powder was also studied [64-67].

SECTION - C

QUANTITATIVE MICROSCOPY

Various quantitative microscopical parameters were studied for the leaves of *Ipomoea pes-caprae*.

STOMATAL NUMBER AND STOMATAL INDEX[68]

Stomatal number

The average number of stomata present in 1 square millimeter area of each surface of a leaf epidermis is termed as stomata lnumber.

Stomatal index

The stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells including stomata, each stoma being counted as one cell.

Determination of stomatal number and stomatal index

To study the stomatal morphology (type of stomata), stomatal number and stomatal index of leaf, the leaf was subjected to epidermal peeling by partial maceration employing the Jeffrey's maceration fluid.

A fragment was transferred in to microscopic slide and the mount of lower and upper epidermis was prepared with a small drop of glycerol solution at one side of the

cover slip to prevent the slide from drying. The slide was examined under 45X objective and 10X eye piece to which a microscopical apparatus was attached. Circle (O) like mark was marked on the drawing paper for each stoma. The average number of stomata/square mm for each surface of the leaf was calculated and their values are tabulated in **table 1.**

For stomatal index, the glycerin mounted leaf peeling as mentioned above was made and circle (O) like mark for each stomata and a cross (X) like mark for each epidermal cells was marked on the drawing paper. The stomatal index was calculated by using the formula,

Stomatal index $\quad S.I = \frac{S}{E+S} \times 100$

Where,

S = Number of stomata in 1 sq mm area of leaf

E = Number of epidermal cells (including trichomes) in the same area of leaf.

VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER [70]

The term vein islet is used to denote the minute area of photo synthetic tissue encircled by the ultimate division of the conducting strands. The number of vein islets per square mm area is called vein- islet number.

Vein termination number may be defined as the number of vein terminations present in one square mm area of the photosynthetic tissue.

Determination of Vein Islets and Vein Terminations

The fragment of leaf lamina with an area of not less than 1 sq mm excluding the midrib and the margin of the leaf was taken. The fragments of leaf lamina were cleared by heating in a test tube containing chloral hydrate solution on a boiling water bath until clear. The cleared fragments were stained with safranin solution and a temporary mount was prepared with glycerol solution. The stage micrometer placed

on the microscopic stage, examined under 10X objective and 10X eye piece and an area of 1 sq mm square was drawn. The cleared leaf piece was placed on the microscope stage, the vein islets and vein terminals included in the square was drawn.

The number of vein islets and terminals within the square including those over lapping on two adjacent sides and excluding those intersected by others two sides were counted. The results obtained in the number of vein islets and terminals in1 sq mm were tabulated in **table 1.**

PALISADE RATIO [71]

Palisade ratio is the average number of palisade cells under one epidermal cell. It is another important criterion for identifications and evaluations for crude drugs. Since it is constant for a plant species which is useful to differentiate the species and does not altered based on geographical variation.

Determination of Palisade Ratio

Epidermal peeling was done by partial maceration by Jeffery's maceration fluid were prepared. A fragment was transferred into a microscopical slide and the mount of upper epidermis was prepared with a small drop of glycerol on one side of the cover slip to prevent the preparation from drying. The same was examined under 45X objective and 10X eye piece. Four adjacent epidermal cells were traced; focusing gently downward to bring the palisade cells into view and sufficient palisade cells to cover the outlined four epidermal cells were then traced. The palisade cells under the epidermal cells were counted and calculate the palisade ratio by using the following formula and the results were tabulated in **table 1.**

Palisade ratio = Avg. number of palisade cells beneath the 4 epidermal cells/4

SECTION - D

POWDER MICROSCOPY AND ANALYSIS

The behavior of the powder with different chemical reagents was carried out as per standard procedure. The observations are presented in **fig 15**.

Fluorescence analysis [78]

The fluorescent analysis of the plant extracts of *Ipomoea pes-caprae* was carried out by treating the sample with various reagents and expose them under day light and UV light (264 and 366 nm) and the observations are tabulated in **table.2.**

SECTION – E

PHYSICO-CHEMICAL PARAMETERS

STANDARDIZATION PARAMETERS

The evaluation of ash values, loss on drying, foreign organic matter and extractive values etc. gives a clear idea about the specific characteristics of crude drug under examination, besides its macro-morphological or cyto-morphological, microscopically nature in both its entire and its powder form. These diagnostic features enable the analyst to know the nature and characteristic of crude drugs and further evaluation of different parameters indicate their acceptability. The procedures recommended in Indian Pharmacopoeia, 1996 and WHO guidelines, 1998 were followed to calculate total ash, water-soluble ash, acid-insoluble ash and loss on drying. The percentages of extractive values for different solvents were also determined for this plant.

Determination of Volatile Oil

Volatile oils are characterized by their odor, oil like appearance and also it has ability to volatilize at room temperature. Chemically they are mixtures of

monoterpenes, sesquiterpenes and their oxygenated derivatives. Volatile oils can be estimated by hydro distillation method.

An accurately weighed 100g of plant material was crushed and introduced in to the flask containing distilled water and glycerol until one third of the plant material was immersed and few pieces of porcelain bits were added. The flask containing liquid was heated until it boils. After 3h, heating was stopped and the collected oil was recorded on the graduated receiver tube. Oil content of the plant material was calculated in mL/100 g of plant materials.

Determination of foreign organic matter[72]

The part of organ or organs other than those specified in the definition or description of the crude drugs is defined as foreign organic matter.

An accurately weighed 100g of air dried coarse drug and spread out in a thin layer. The sample drug was inspected with the unaided eye or with the use of 6X lens and the foreign organic matter was separated manually as completely as possible and weighed. The percentage of foreign organic matter was calculated with reference to the weight of the drug taken. The result is presented in **table 3.**

Determination of Moisture Content (Loss on Drying)

An accurately weighed 10 g of coarsely powdered drug was placed in a tarred evaporating dish. Then the dish was dried at 105^{o}C for 5 h and weighed. The drying and weighing was continued at one hour intervals until the difference between the two successive weighing is not more than 0.25 %. The loss on drying was calculated with reference to the amount of powder taken. The readings are tabulated in **table 3.**

Determination of Ash values[73]

Ash Content

The residue remaining after incineration is the ash content of crude drug, which simply represents inorganic salts naturally occurring in the drug or adhering to it or deliberately added to it as a form of adulteration.

Determination of Total Ash

An accurately weighed 3 g of air dried coarsely powdered drug was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450^{o}C, until free from carbon then allowed to cool and weighed. The percentage of ash was calculated with reference to the air dried drug.

Determination of Acid Insoluble Ash

The total ash obtained from the previous procedure was mixed with 25 mL of 2 M hydrochloric acid and boiled for 5 min in a water bath, and then the insoluble matter was collected in an ashless filter paper and washed with hot water, dried and ignited for 15 min at a temperature not exceeding 450^{o}C, cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Determination of Water Soluble Ash

The total ash obtained from the previous procedure was mixed with 25 mL of water and boiled for 5 min in a water bath, and then the insoluble matter was collected in an ashless filter paper and washed with hot water, dried and ignited for 15 min at a temperature not exceeding 450^{o}C, cooled in desiccators and weighed. The insoluble matter was subtracted from the weight of the total ash; the difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

The values in respect of the total ash values, acid insoluble ash, water soluble ash and water insoluble ash are tabulated in **table 3**.

DETERMINATION OF EXTRACTIVE VALUES [74]

Determination of ethanol soluble extractive

An accurately weighed 5 g of the air dried coarsely powdered drug was macerated with 100mL of ethanol in a closed flask for 24 h, shaking frequently during the first 6 h and allowed to stand for 18 h. Thereafter filtered rapidly, taking precautions against loss of ethanol. Then evaporate 25 mL of the filtrate to dryness in a tarred flat bottomed shallow dish dry at 105^{o}C and weighed. The percentage of ethanol soluble extractive was calculated with reference to the air dried drug.

Determination of water soluble extractive, hexane soluble extractive and petroleum soluble extractive, methanol soluble extractive, chloroform soluble extractive and ether soluble extractive values

The procedure adopted for ethanol soluble extractive value was followed for the determination of water soluble extractive, hexane soluble extractive and petroleum soluble extractive, methanol soluble extractive, chloroform soluble extractive and ether soluble extractive values and the results are presented in **table 4**

Determination of Foaming Index

Plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of foaming index.

An accurately weighed 1 g of the coarse plant material was transferred into a 500 mL conical flask containing 100 mL of boiling water. The flask was maintained at moderate boiling for 30 min. The solution was cooled and filtered into a 100 mL volumetric flask and sufficient distilled water was added to dilute to volume. The decoction was poured into 10 stoppered test tubes in successive portions of 1 mL,

2mL, etc. up to 10 mL, and the volume of the liquid in each tube was adjusted with water up to 10 mL. The tubes were stoppered and shaken in a length wise motion for 15 sec (two shakes/sec) and allowed to stand for 15 min. The height of foam was measured. If the height of the foam in every tube was less than 1cm the foaming index was less than 100. If a height of foam of 1 cm was measured in any test tube, the volume of the plant material decoction in this tube (a) was used to determine the index. If this tube was first or second tube in a series, an intermediate dilution was prepared in a similar manner to obtain a more precise result.

If the height of the foam was more than 1cm in every tube, the foaming index was over 1000. In this case, the determination was repeated using a new series dilution of the decoction in order to obtain a result. The foaming index was calculated using the following formula 1000/A where A was the volume in mL of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm was observed. The result obtained is presented in **table 4.**

Determination of Swelling Index[75]

Swelling index is the volume in mL taken up by the swelling of 1 g of plant material under specified conditions. Its determination is based on the addition of water. Using a glass stoppered measuring cylinder, the material is shaken repeatedly for 1 h and then allowed to stand for required period of time. The volume of the mixture (in ml) is read.

About 1 g of weighed powder was introduced in to a 25 mL glass stoppered measuring cylinder, and 25 mL of water was added and the mixture was shaken thoroughly every 10min for 1 h and allowed to stand for 3 h at room temperature. The volume in mL occupied by the plant material including the sticky mucilage was measured. The mean value of the individual determination was calculated related to 1 gm of plant material and the result is tabulated in **table 4**.

PHYTOCHEMICAL ANALYSIS

PART - II

PHYTO CHEMICAL STUDIES

Phytochemistry deals with natural product organic chemistry and plant biochemistry. It also deals with a variety of secondary metabolites that are produced by plants, their chemical structures, biosynthesis, metabolism, natural distribution and biological functions. This chapter deals with isolation and identification of phytoconstituents of this plant.[80]

Collection and authentication

The leaves of *Ipomoea pes-caprae* was collected in and around Ramnad and authenticated by taxonomist. The shade dried leaves were powdered and then subjected to the following preliminary phytochemical studies.

SECTION- A

PRELIMINARY PHYTOCHEMICAL SCREENING

All the extracts were subjected to qualitative chemical analysis. The various chemical tests were performed on the extracts for the identification of sterols, terpenoids, flavones, anthraquinones, sugars glycosides, alkaloids, quinones, phenols and tannins. The results were tabulated in **table 5& 6**.

1. TEST FOR STEROLS[81]

The powdered leaf was first extracted with petroleum ether and evaporated toa residue. Then the residue was dissolved in chloroform and tested for sterols.

a. SALKOWSKI'S TEST

A few drops of concentrated sulphuric acid was added to the above solution, shaken well and set aside. The lower chloroform layer of the solution turned red in color indicating the presence of sterols.

b. LIEBERMANN – BURCHARD'S TEST

To the chloroform solution a few drops of acetic anhydride and 1 ml of concentrated sulphuric acid were added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring was formed. The upper layer turned green indicating the presence of sterols.

2. TEST FOR CARBOHYDRATES

a. MOLISCH'S TEST

The extract of the powdered leaf was treated with 2-3 drops of 1% alcoholic α-naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. A purple color indicating the presence of carbohydrates.

b.FEHLING'S TEST

The extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Red precipitate was obtained indicating the presence of free reducing sugars.

c .BENEDICT'S TEST:

The extract of the powdered leaf was treated with equal volume of Benedict's reagent. A red precipitate was formed indicating the presence of reducing sugar.

3.TEST FOR PROTEINS

a. MILLON'S TEST

A small quantity of acidulous – alcoholic extract of the powdered drug was heated with Millon's reagent. White precipitate turned red on heating indicate the presence of proteins.

b. BIURET TEST

To one portion of acidulous – alcoholic extract of the powdered drug one ml of 10% sodium hydroxide solution and one drop of dilute copper sulphate solution were added. Violet color was obtained indicating the presence of proteins.

4. TEST FOR ALKALOIDS

About 2 gm of the powdered material was mixed with 1gm of calcium hydroxide and 5 mL of water into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200 mL of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To this 5 mL of dilute hydrochloric acid was added followed by 2 mL of each of the following reagents.

a) MAYER'S TEST:

A small quantity of the extract was treated with Mayer's reagent. Cream colour precipitate indicates the presence of alkaloids.

b) DRAGEN DORFF'S TEST:

A small quantity of the extract was treated with Dragendorff's reagent. Orange brown precipitate indicates the presence of alkaloids.

c) WAGNER'S TEST:

A small quantity of extract was treated with Wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.

d) HAGER'S TEST:

A small quantity of extract was treated with Hager's reagent. Yellow precipitate indicates the presence of alkaloids.

e) TEST FOR PURINE GROUP (MUREXIDE TEST)

The residue obtained after the evaporation of chloroform was treated with 1mL of hydrochloric acid in a porcelain dish and 0.1 gm of Potassium chlorate was added and evaporated to dryness on water bath. Then the residue was exposed to the vapour of dilute ammonia solution. No purple Color was obtained indicating the absence of purine group of alkaloids.

5. TEST FOR GLYCOSIDES

a) BORNTRAGER'S TEST

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly. Ammoniacal layer showed the pink colour indicating the presence of anthraquinone glycosides.

b) MODIFIED BORNTRAGER'S TEST

About 0.1 g of the powdered drug was boiled for 2 minutes with dilute hydrochloric acid and few drops of ferric chloride solution, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract. Pink color was observed in ammoniacal layer showing the presence of anthraquinone glycosides.

c) TEST FOR CARDIAC GLYCOSIDES(FOR DEOXYSUGAR)

i) KELLER KILIANI TEST

About 1 g of the powdered leaf was boiled with 10 ml of 70 % alcohol for 2 minutes, cooled and filtered. To the filtrate 10 mL of water and 5 drops of solution of lead subacetate were added and filtered, evaporated to dryness. The residue was dissolved in 3 mL of glacial acetic acid. To these 2 drops of ferric chloride solution

was added. Then 3 mL of concentrated sulphuric acid was added to the sides of the test tube carefully and observed. No Reddish brown layer was observed indicating the absence of deoxysugars of cardiac glycoside

d) TEST FOR CYANOGENETIC GLYCOSIDES

Small quantity of the powder was placed in a stoppered conical flask with just sufficient water, to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place. No brick red color was produced on the paper indicating the absence of cyanogenetic glycosides.

6.TEST FOR SAPONINS

About 0.5g of the powdered drug was boiled gently for 2 minutes with 20 mL of water and filtered while hot and allowed to cool. 5 mL of the filtrate was then diluted with water and shaken vigorously. Frothing was produced indicating the presence of saponins.

7. TEST FOR TANNINS

A small quantity of the powdered drug was extracted with water. To the aqueous extract few drops of ferric chloride solution was added. Bluish black color was produced indicating the presence of tannins.

8. TEST FOR FLAVONOIDS

a. SHINODA'S TEST

A little of the powdered drug was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added. Boiled for five minutes. Red colour was obtained indicating the presence of flavonoids.

b. ALKALI TEST

To the small quantity of test solution 10% aqueous sodium hydroxide Solution was added. Yellow orange color was produced indicating the presence of flavonoids.

c. ACID TEST

To the small quantity of test solution, few drops of concentrated sulphuric acid were added. Yellow orange colour was obtained indicates the presence of flavonoids.

9. TEST FOR TERPENOIDS

A little of the powdered leaf was extracted with chloroform and filtered. The filtrate was warmed gently with tin and thionylchloride. Pink color solution appeared which indicated the presence of terpenoids.

10. TEST FOR THE PRESENCE OF VOLATILE OIL

Weighted quantity (250 gm) of fresh leaves were extracted and subjected to hydro distillation using volatile oil estimation apparatus. No volatile oil was obtained indicating the Absence of volatile oil.

11. TEST FOR MUCILAGE

Few ml of aqueous extract prepared from the powdered crude drug was treated with ruthenium red. No Red colour was produced indicating the absence of mucilage.

SECTION – B

QUANTITATIVE ESTIMATION OF PHYTO-CONSTITUENTS

Preparation of ethanolic extract of *Ipomoea pes-caprae*

The shade dried and coarsely powdered leaves of *Ipomoea pes-caprae* was defatted with petroleum ether (60-80˚c) for three days by triple maceration. The defatted marc was extracted with 70% ethanol by triple maceration and filtered. The filtrate was concentrated under reduced pressure to obtain a solid residue which was dark green in colour.

TOTAL PHENOLIC DETERMINATION[82-84]

Principle

Total phenolic content of the various concentrations of extract of *Ipomoea pes-caprae*byFolin-ciocalteu reagent method.

All the phenolic compounds are oxidized by the Folin-Ciocalteu Reagent. This reagent, which is reduced during oxidation of the phenolic substances, into a mixture of blue molybdenum and tungsten oxides.

The blue colour produced has a maximum absorption at about 750-760 nm. The absorption is proportional to the quantity of oxidized phenolic compounds.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents required

a) FolinCiocalteu Reagent (1N)

The Folin-ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate. Commercially available Folin-ciocalteu reagent (2N) was diluted with an equal volume of distilled water. The resultant solution was kept in a brown color bottle and stored in refrigerator at 4 ºc.

b)Sodium carbonate solution (10%)

Sodium carbonates 350 gm was dissolved & makes the volume up to 1000 mL& heat the solution at 70-80°c, then cool & filter with glass wool & keep it overnight.

c)Standard gallic acid solution.

Procedure

About 1 mL (1mg/ml and 0.5 mg/mL) of ethanolic extracts of *Ipomoea pes-caprae* 0.5 mL of Folin-ciocalteu reagent (1N) were added and allowed to stand for

15 minutes. Then 1 mL of 10% sodium carbonate solution was added to the above solution. Finally the mixtures were made up to 10 mL with distilled water and allowed to stand for 30 minutes at room temperature and total phenolic content was determined spectrophotometrically at 760nm wavelength.

The calibration curve was generated by preparing Gallic acid at different concentration (2, 4, 6, 8 and 10 µg/mL). The reaction mixture without sample was used as blank. Total phenolic content of IPC extract is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g). The results are tabulated in **table 7** and the calibration graph was presented at **Fig 16.**

TOTAL FLAVONOID DETERMINATION[85-87]

Principle

The aluminum chloride colorimetric technique was used for estimation of total flavonoid estimation. Aluminum ions form stable complexes with C_4 keto group and either to C_3 or C_5 hydroxyl groups of flavones and flavonols in acidic medium. It also forms acid labile complexes with ortho hydroxyl groups in the A or B rings of flavanoids. These complexes showed a strong absorption at 415 nm which is used for the estimation of flavonoids.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents required

10% aluminum chloride

1M potassium acetate

Procedure

1mL of ethanolic extracts of *Ipomoea pes-caprae*, 0.1 mL of aluminum chloride solution, 0.1 mL of potassium acetate solution and 2.8 mL of ethanol were

added and the final volume was then made up to 5 mL with distilled water. After 20 min the absorbance was measured at 415 nm.

A calibration curve was constructed by plotting absorbance reading of quercetin at different concentrations. The sample without aluminium chloride was used as a blank. The total flavonoid content in the extract was expressed as milligrams of quercetin equivalent per gram of extract and the results were tabulated in **table 8**. **(Fig.17)**

TOTAL TANNIN DETERMINATION[88,89]

Principle

Total tannin content of extract was determined by Folin Denis reagent method.

Tannin like compounds reduces phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution. The intensity of which is proportional to the amount of tannins and it was estimated by spectrophotometer at 700 nm.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents required

a) Folin Denis Reagent (sodium tungstate 100 g and phospho molybdic acid 20 gm were dissolved in distilled water 750 mL along with phosphoric acid 50 mL. The mixture was refluxed for 2 hours and volume was made up to 1 litre with distilled water)
b) Sodium carbonate solution (10%)
c) Standard tannic acid solution.

Procedure

0.2 mL of (1 mg/mL) ethanolic extract of *Ipomoea pes-caprae*, was made upto1 mL with distilled water. Then add 0.5 mL of Folin Denis reagent and allowed to stand for 15 mins, then 1 mL of sodium carbonate solution was added to the mixture and it was made up to 10 mL with distilled water. The mixture was allowed to stand for 30 mins at room temperature and the tannin content was determined spectrophotometrically at 700 nm.

The calibration curve was generated by preparing tannic acid at different concentration (4, 8, 12, 16 and20 µg/mL). The reaction mixture without sample was used as blank. The total tannin content in the leaf extract was expressed as milligrams of tannic acid equivalent per gm of extract. The results were tabulated in **table 9**. **(Fig 18)**

SECTION –C

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Analysis and quantification of kaempferol in *Ipomoea pes-caprae* extract by HPLC

HPLC studies of IPC extract was carried out in order to identify and quantity the active principles present in the extract.

Materials and methods[91,92]

Instrument	-	Agilent technologies 1220 infinity LC (USA)
Column	-	Agilent Zorbax SB – C 18
Flow rate	-	1.2 mL / min
Injection volume	-	10 µL
Standard	-	Kaemperol (5mg/ 100mL of methanol)
Sample	-	*Ipomoea pes-caprae* (0.1g/100 mL of methanol)

UV Detector - 270 nm

Temperature - 30 ˚c

Software - Open LAB,CDS chem station work station VL

Mobile phase - The mobile phase was prepared using 1 %(v/v) tetrehydrofuran in deionized distilled water adjusting to pH 3.0 with H_3PO_4. The flavonoids were separated using the following gradient; 30 %(v/v) CH_3CN (acetonitrile) for 8 mins,100 % CH_3CN for 3 mins,100 % CH_3CN for 4 mins.

Linearity graph was generated by plotting the different concentration of the standard in the X axis (3.81, 7. 61, 11.42, 15.23, 19.04 µg/ mL**)** and the area of the peak in Y axis.

The linear regression equation was calculated as y = 35.3123x- 5.7080, R^2 = 1.0000.The result of the standard and sample chromatogram are presented in **Fig19-25.**

PHARMACOLOGICAL SCREENING

PART III- PHARMACOLOGICAL SCREENING

SECTION- A

IN VITRO ANTIOXIDANT ACTIVITY

Method 1 ; Determination of scavenging activity against hydrogen peroxide[93-95]

Principle

The radical scavenging activity against hydrogen peroxide of plant extract was determined by using the method of Janani*et al* (2011). The principle is based on the capacity of the extract to decompose the hydrogen peroxide to water. H_2O_2 in the presence of **O_2 -** can generate highly reactive hydroxyl radicals via the metal, the scavenging of H_2O_2 in cells is critical to avoid oxidative damage. Thus, the scavenging of hydrogen peroxide is an important antioxidant defence mechanism.

$$Fe2+ + H2O2 \longrightarrow Fe3+ + OH + OH-$$

The decomposition of hydrogen peroxide to water involves the transfer of electrons as in equation

$$H2O2 + 2H+ + 2e- \longrightarrow 2H2O$$

Reagents

6% hydrogen peroxide diluted with water in the ratio of 1:10

0.1M, pH 7.4 phosphate buffer

Procedure

To 1 mL of test solutions of different concentrations, 3.8 mL of 0.1 M phosphate buffer solution (pH 7.4) and then 0.2 mL of hydrogen peroxide solution were added. The absorbance of the reaction mixture was measured at 230 nm after 10 min. The reaction mixture without sample was used as blank. Sample blank was also prepared without reagents. Ascorbic acid was used as standard. The percentage inhibition of hydrogen peroxide was calculated using the formula

$$\% \text{ inhibition} = (A_{control} - A_{sample}) / A_{control} \times 100$$

The concentration of the sample required for 50 % reduction in absorbance (IC_{50}) was calculated using linear regression analysis. The results obtained are presented in **table 10.** **(Fig. 26)**.

Method 2; Diphenylpicrylhydrazyl (DPPH) method[96-98]

Principle

DPPH is a stable free radical with a distinctive ESR signal. Its reaction with antioxidants can be followed by the loss of absorbance at 517 nm. It is widely accepted that DPPH accept an electron or hydrogen radical and become a stable diamagnetic molecule. Due to its odd electron, the ethanol solution of DPPH (purple colour solution) shows a strong absorption at 517 nm. DPPH radicals react with suitable reducing agents where the pairing of electrons takes place and the solution loses colour stochiometrically with the number of electrons taken up.

$$DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot}$$

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

0.1mM diphenylpicryl hydrazyl in ethanol

Procedure

A stock solution of DPPH was prepared in ethanol (4 mg/100 ml). To the 1 mL of test samples of different concentrations 4 mL of DPPH was added. Control without test compound was prepared in an identical manner. Blank was prepared in the similar way, where DPPH was replaced by ethanol. The reaction was allowed to be completed in the dark for about 30 min. Then the absorbance of test mixtures was

read at 517 nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical. Vitamin C was used as standard.

The percentage scavenging was calculated using the formula

$$\% \text{ inhibition} = (A_{control} - A_{sample}) / A_{control} \times 100$$

The concentration of the sample required for 50 % reduction in absorbance (IC_{50}) was calculated using linear regression analysis. The results obtained are presented in **table 11**. **(Fig 27)**

Method 3; Total antioxidant activity by phosphomolybdenum method[99,100]

Principle

The total antioxidant activity of the extract was evaluated by phosphomolybdenum method. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and by the subsequent formation of green phosphate Mo (V) complex at acidic pH which has a maximum absorption at 695 nm. This method is routinely used to determine total antioxidant activity of samples.

$$Mo^{6+} \rightarrow Mo^{5+}$$

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

0.6M sulphuric acid

28mM sodium phosphate

4mM ammonium molybdate

Procedure

An aliquot of 0.3 mL of different concentrations of sample solutions was combined with 2.7 mL of the reagent solution (H_2SO_4, sodium phosphate and ammonium molybdate). In case of blank, 0.3 mL of methanol was used in place of

sample. The tubes were incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank. The standard vitamin C was treated in a similar manner. The antioxidant activity was expressed as equivalents of Vitamin C (µg/g). The results are tabulated in **table 12**.(**Fig 28)**

Method4 : Reducing power assay [101,102]

Principle

Reducing power assay is a spectrophotometric method and is based on the principle that increases in absorbance of the reaction mixture indicates the increases in the reducing power of the sample. Antioxidant activity may be due to a variety of mechanism viz., the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the reductive capacity and free radical scavenging. The assay is based on the reduction of ferric in potassium ferricyanide to ferrous to form potassium ferrocyanide by the sample and the subsequent formation of Prussian blue colour with ferric chloride. The absorbance of the blue complex is measured at 700 nm.

Potassium ferricyanide+ Ferric chloride

⇩ Antioxidant

Potassium ferrocyanide + Ferrous chloride

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Materials required

Ascorbic acid

1 % w/v Potassium ferricyanide

10 % w/v Trichloro acetic acid

0.2 M, Phosphate buffer (pH 6.6)

0.1% w/v Ferric chloride

Procedure

The reducing power ability of plant extracts was screened by assessing the ability of the test extract to reduce $FeCl_3$solution as mentioned by Oyaizu *et al.*, (1986). 0.1 to 0.5 mL of plant extract solution (1 mg/mL) was mixed with 0.75 Ml of phosphate buffer and 0.75 mL of 1 % potassium ferricyanide [$K_3Fe(CN_6)$] and incubated at 50^oC for 20min.0.75 mL of 1 % trichloro acetic acid was added to the mixture and allowed to stand for 10min. The whole mixture was then centrifuged at 3000 rpm for 10min. Finally 1.5 mL of the supernatant was removed and mixed with 1.5 mL of distilled water and 0.1mL of 0.1 %ferric chloride solution and the absorbance was measured at 700 nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer was used as blank solution. The results obtained are presented in **table 13.(fig 29)**

SECTION - B

EFFECT OF ETHANOLIC EXTRACTS OF *Ipomoea pes-caprae* ON VINCRISTINE INDUCED NEUROPATHIC PAIN IN RATS [103-110]

Neuropathic pain associated with peripheral nerve injury is characterized by the sensory abnormalities such as unpleasant and abnormal sensation (dysesthesia) an increased response to painful stimuli (hyperalgeia) and pain in response to stimuli that does not normally provoke pain(allodynia).[5]

MATERIALS AND METHODS

Plant meterial

The fresh leaves were collected at Ramnad, Tamil Nadu and India. The plant material was authenticated by taxonomist. After authentication, fresh leaves of

ipomoea pes-caprae was cleaned thoroughly with distilled water and dried under shade. The shade-dried leaves were pulverized in a mechanical grinder. The powdered plant material passed through a sieve.(sieve no. 10/40).

Chemicals and drugs

Vincristine sulfate was obtained from Chandra Bhagatpharma Pvt. Ltd., Mumbai, India. Pregabalin was procured from Glenmark and all the chemicals used were of analytical grade.

Animals

Wistar albino rats, weighing 200-300 g, were employed in the present study. The rats were exposed to 12 h light-dark cycles. The experimental protocol was duly approved by the Institutional Animal Ethics Committee.

Induction of peripheral neuropathy

Peripheral neuropathy was induced in rats by administration of vincristine sulfate (50 µg/kg; *i.p*. daily) for 10 consecutive days. Behavioral test were assessed on different days. i.e. 0,1,3,6,9,12,15,18 and 21st.

EXPERIMENTAL PROTOCOL

Eight groups, each comprising six Wistarrats were employed in the present study.

Group I (Normal control group)

Rats were not subjected to administration of vehicle and vincristine and were kept for 24 days. Behavioral tests such as hot plate test, acetone drop test and tail immersion test were employed to assess nociceptive threshold of the hind paw on different day's i.e. 0,1,3,6,9,12,15,18&21st .All the animals were sacrificed according to CPCSEA guidelines at the end of the 21st day. Sciatic nerve of the animals were isolated and homogenated to estimate biochemical markers such as TBARS, reduced

glutathione, total protein and total calcium levels. Histopathological studies also carried with the distal portion of the sciatic nerve.

Group II (vincristine 50 µg/kg,*i.p*)

Vincristine (50 µg/kg, *i.p)* was administered to the rats for 10 consecutive days. Behavioral tests were assessed as described in group I.

Group III (IPC extract 200 mg/kg treated group)

IPC extract (200 mg *p.o)* was administered two hours before each vincristine injection (vincristine was administrated daily for 10 days; 50 µg/mg, *i.p*) for 10 consecutive days. Behavioral tests were assessed as mentioned in group I.

Group IV (IPC extract 300 mg/kg treated group)

IPC (300 mg/kg *p.o)* was administered two hours before each vincristine injection

(vincristine was administrated daily for 10 days; 50 µg/mg, *i.p*) for 10 consecutive days. Behavioral tests were assessed as mentioned in group I.

Group V (Pregabaline 10mg/kg, treated group)

Pregabalin (10 mg/kg *p.o)* was administered orally two hours before each vincristine injection (vincristine was administered once in three days) for 14 consecutive days. Behavioral tests were assessed as mentioned group I.

SENSORY BEHAVIORAL ASSESMENT

Hot plate test[103]

Heat thermal sensitivity of the hind paw was assessed by using standard procedure for assessing the degree of noxious thermal sensation. The rats were placed on the top of a preheated (52± 0.5˚ c) hot plate. The hind paw withdrawal response was noted in seconds. The cut-off time of 20 seconds was maintained. **(fig 30).**

Tail immersion test[104]

Tail immersion test was carried out to assess the spinal heat thermal sensitivity. Tip of the rat's tail was immersed in heat noxious temperature (52± 0.05˚c) till the tail was withdrawn. Thermal heat hyperalesia was assessed by the tail withdrawl response, noted in seconds. The cut off time of 10 seconds was maintained.**(fig 31)**

Acetone drop test (Paw cold-allodynia)[105]

Cold-allodynia of the hind paw was assessed using acetone drop method using standard procedure with slight modification, for evaluating the reactivity to non-noxious cold chemical stimuli. The rats were placed on the top of a wire mesh grid, allowing access to the hind paws. Acetone (0.1 ml) was sprayed on the plantar surface of hind paw of rat and time taken for withdrawing the hind paw from the mesh surface was noted. The cut off time of 60 seconds **(fig 32)**

BIOCHEMICAL ESTIMATION OF MARKERS OF OXIDATIVE STRESS

All the groups of animals were sacrificed after 21stday by cervical dislocation and the sciatic nerve was isolated immediately and used for the biochemical estimation. The distal portion of the nerve, which was used for histopathological study. Freshly excised sciatic nerve homogenate (10 %) was prepared with 0.1 M TrisHcl buffer (P^H-7.4) and the homogenate was kept in ice water for 30 min and centrifuged at 4˚c (2000 g,10 min).The supernatant of homogenate was separated and which was used to estimate following biochemical markers.

Estimation of total protein content[106]

The protein concentration was estimated according to the standard procedure[112] using bovine serum albumin as a standard. The absorbance was determined spectrophotometrically at 750 nm.

Estimation of total calcium[107,108]

Total calcium level was estimated in sciatic nerve according to the standard procedure. Total calcium level was estimated in sciatic nerve. The sciatic nerve homogenate was mixed with 1 mL of trichloroaceticacid (4 %) as in ice cold condition and centrifuged at 1500 g for 10 mins. The clear supernatant was used for the estimation by atomic emission spectroscopy at 556 nm.

Estimation of reduced glutathione[109]

Equal quantity of sciatic nerve tissue homogenate was mixed with 10 % tricholoroacetic acid and the mixture was centrifuged to separate proteins. To 0.01 mL of this supernatant, 2 mL of phosphate buffer(PH-8.4). 0.5 mL of 5'5 dithiobis (2-nitrobenzoic acid) and 0.4mL of distilled water were added. Mixture was vortexed and the absorbance was taken at 415 nm with 15 mins. The concentration of reduced glutathione was expressed as µg/mg of proteins.

Estimation of TBARS

The thiobarbituric acid reactive substance (TBARS) level was estimated as per the standard procedure. To each test tube,0.5 mL of supernatant,0.5 mL normal saline, 1 mL of 20 % tricholoroacetic acid (TCA) AND 0.25 mL of TBA reagent (200 mg of thiobarbituric acid in 30 mL distilled water and 30 mL of acetic acid) were added. The test tubes were kept for boiling at 95˚ c for one hour. To each test tube, 3 mL of n-butanol was added and mixed well. These test tubes were centrifuged at 3000 rpm for 10 minutes. The separated butanol layer was collected and read in a spectrophotometer against blank at 535 nm. Concentration of thiobarbituric reactive substance was expressed in terms of nmolmalondialdehyde per mg of protein. The results are presented to **table 14.**

Histopathology studies[110]

Samples of sciatic nerve were stored in fixative solution (10 % formalin) and cut in to 4 µm thickness. Staining was done by using hematoxylin and eosin as described by standard procedure. Nerve sections were analysed qualitatively with a light microscope (450x) for axonal degeneration.**(Fig 33)**

Statistical analysis

All the results were expressed as mean ± standard error of means (SEM). The data from the behavioral results were statistically analysed by two-way analysis of variance followed by bonferroni's post hoc-test by using graph pad prism v.5.0 software. The data from the biochemical results were statistically analysed by one-way ANOVA followed by Tukey's multiple range tests. $p < 0.05$ was considered to be statistically significant.

SECTION - C

IN VITRO MEMBRANE STABILISATION STUDY [111-113]

Principle

The method *in vitro* membrane stabilization activity assay was carried out according to the established and standard procedure. When RBCs are subjected to heat and treatment with hyposaline, they release haemoglobin which has a maximum absorbance at about 560 nm. The capacity of the extract to reduce hyposaline and heat induced lysis is basis of the assay.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

0.2M sodium phosphate buffer (pH 7.4)

0.36% w/v hyposaline

10%v/v HRBC suspension in isosaline

Preparation of HRBC suspension in isosaline

The human erythrocytes suspension was used for the in vitro membrane stabilization assay. Blood was collected from healthy volunteers who had not consumed any NSAIDs for two weeks prior to the experiment. The blood was mixed with equal volume of Alsever solution (2% dextrose, 8.0 % sodium citrate, 0.5 % citric acid and 0.42 % sodium chloride) and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10 % v/v erythrocyte suspension in isosaline was prepared.

Procedure

The assay mixture consist of 2 mL of hyposaline and 1mL of phosphate buffer and varying volumes of the extract (0.1 to 0.5 mL) and 0.5 mL of HRBC suspension in isosaline, then the final volume were made up with isosaline up to 4.5 mL. The control was prepared as mentioned above except the drug was omitted, while drug control was also prepared similarly but without HRBC suspension. The reaction mixture was incubated at 56ºC for 30min in a water bath, then the tube was cooled under running water. Then the absorbance of the released hemoglobin was measured at 560 nm. Diclofenac 50 µg/mL was used as a reference standard. The percentage of membrane stabilization activity of the compounds were determined by the formula

% membrane stabilization = $[A_{control} - (A_{test} - A_{product\ control})] / A_{control} \times 100$

$A_{control}$ - Absorbance in control

A_{test} - Absorbance in test

$A_{product\ control}$ - Absorbance in product control

The results obtained for *in vitro* membrane stabilization effect is presented in **table 15.(fig 34)**

RESULTS AND DISCUSSION

CHAPERT – 6

RESULTS AND DISCUSSION

PART – I PHARMACOGNOSTIC EVALUATION

Section A – Macroscopic studies of leaves

Colour	-	Dark green colour
Odour	-	Characteristic odour
Texture	-	Coarse
Venation	-	Pinnate
Leaf arrangement	-	Alternate
Leaf type	-	Simple
Margin	-	Lobed
Shape	-	Eliptic (oval)

Section – B Microscopic studies of the leaves

Leaves

The leaves has broad and thick midrib and thin lamina. The midrib consist of wide adaxial part, bowel shaped and thick abaxial part. It is 1.1 mm, adaxial cone is 150 x 250 µm in size and the abaxial part is 1.8 mm wide.**(Fig 11.1)**

The epidermal layer around the midrib is intact and includes small, thick walled- squanish cells. The ground tissue is parenchymatous; the cells are angular or circular and compact. The cells in the adaxial cone are collenchymatous. The palisade zone extends up to the lateral shoulders of the adaxial cone.

The vascular strand is shallowly saucer shaped. It is 300 µm thick and 1 mm broad.The vascular strand consists of three or four separate units of bi-collateral bundles. In each bundle occur short, radial lines of three or four xylem elements. The elements are wide, angular and thick walled. The Metaxylem elements are 40 µm wide.

FIG 4: HERBERIUM OF *Ipomoea pes-caprae*

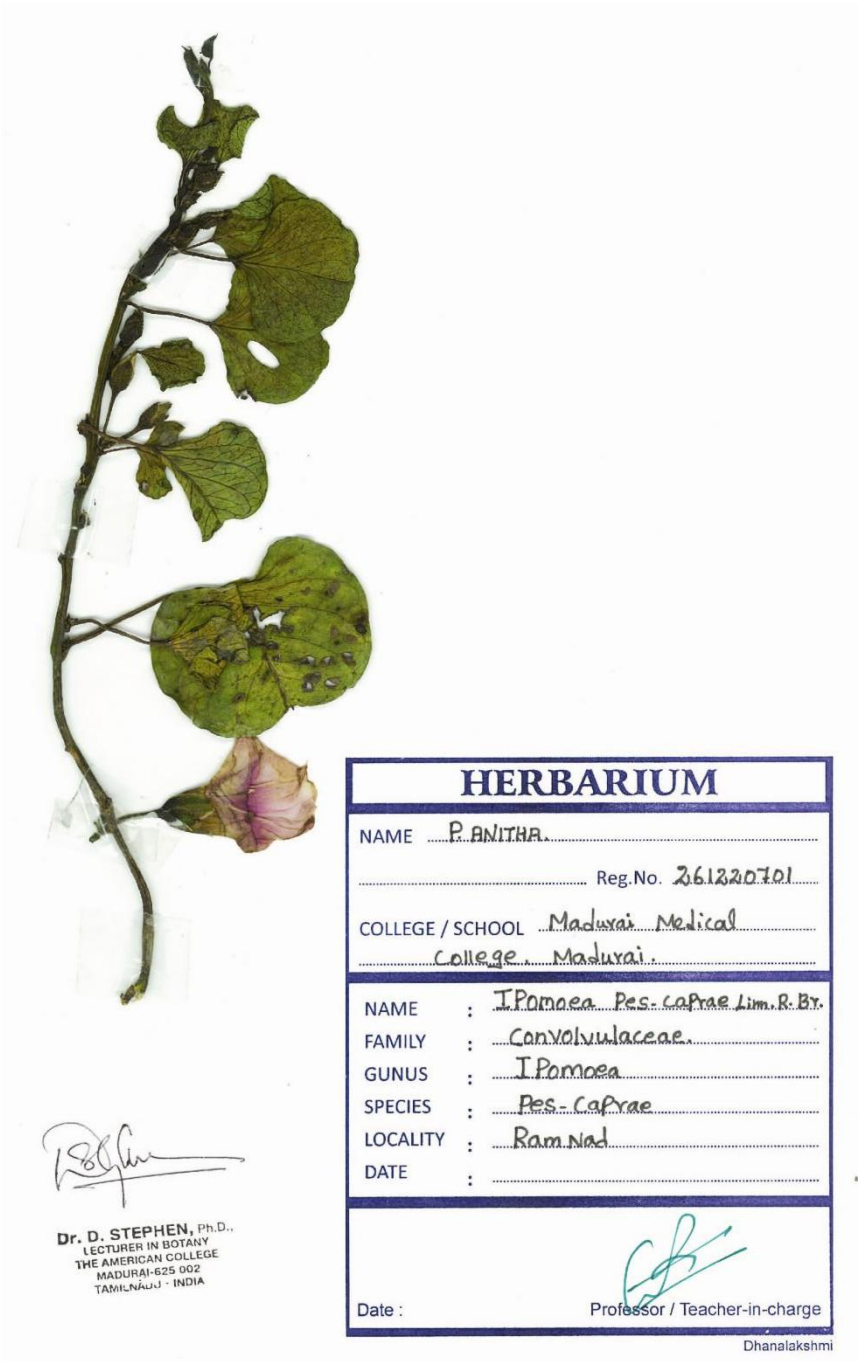


FIG 5: HABIT AND HABITAT OF *Ipomoea es-capare*

FIG 6:BUDS OF *Ipomoea pes-capare*

FIG 7:FLOWER OF *Ipomoea pes capare*

FIG 8: LEAVES OF *Ipomoea pes-caprae*

DORAL LEAF **VENTRAL LEAF**

FIG 9: SEEDS OF *Ipomoea pes-caprae*

FIG 10: STEM OF *Ipomoea pes caprae*

The phloem elements are small, thick walled and darkly stained. They are present in both adaxial and abaxial sides of the vascular bundle. The abaxial phloem units are thick and circular masses. The abaxial units are small clusters.**(fig 12.1,12.2)**

Lamina

The lamina is bifacial (heterofacial), amphistomatic and mesomorphic. The lamina is 340 µm thick. The adaxial epidermis is thin and the cells are narrow and cylindrical. The abaxial epidermis is thick and it consists of rectangular- oblong cells. The stomata are slightly raised above the epidermal surface. The mesophyll tissues are differentiated into adaxial band of vertical filaments; the filament is two or three cells and loosely arranged. The spongy parenchyma cells are short and cylindrical and forms a reticulate system of aerenchyma **(fig11.2).**

Epidermal cells and stomata

The stomata and epidermal cells are observed in the paradermal (parallel to the epidermal surface) sections. The epidermal cells are polygonal with straight, thick anti clinal walls. Stomata of both adaxial and abaxial sides are paracytic type **(fig 13.1)**. The stoma has two subsidiary cells, one or either side and parallel guard cells **(fig 13.2)**. The guard cells are 20 x 40 µm in size.

Glandular trichomes

Peltate type of glandular trichomes are common on the epidermal layer. The gland has a short one-celled stalk and circular 8- celled flat head. The gland is 30 µm in diameter.**(fig 13.3)**

Venation

The venation is densely reticulate. The secondary and tertiary veins are uniformly thin. The vein islets are wide and poly hedral in outline. Veinlet terminatios are either simple and unbranched; more commonly they are branched forming

FIG. 11.1

T.S. of Leaf of *Ipomoea pes-caprae* through midrib

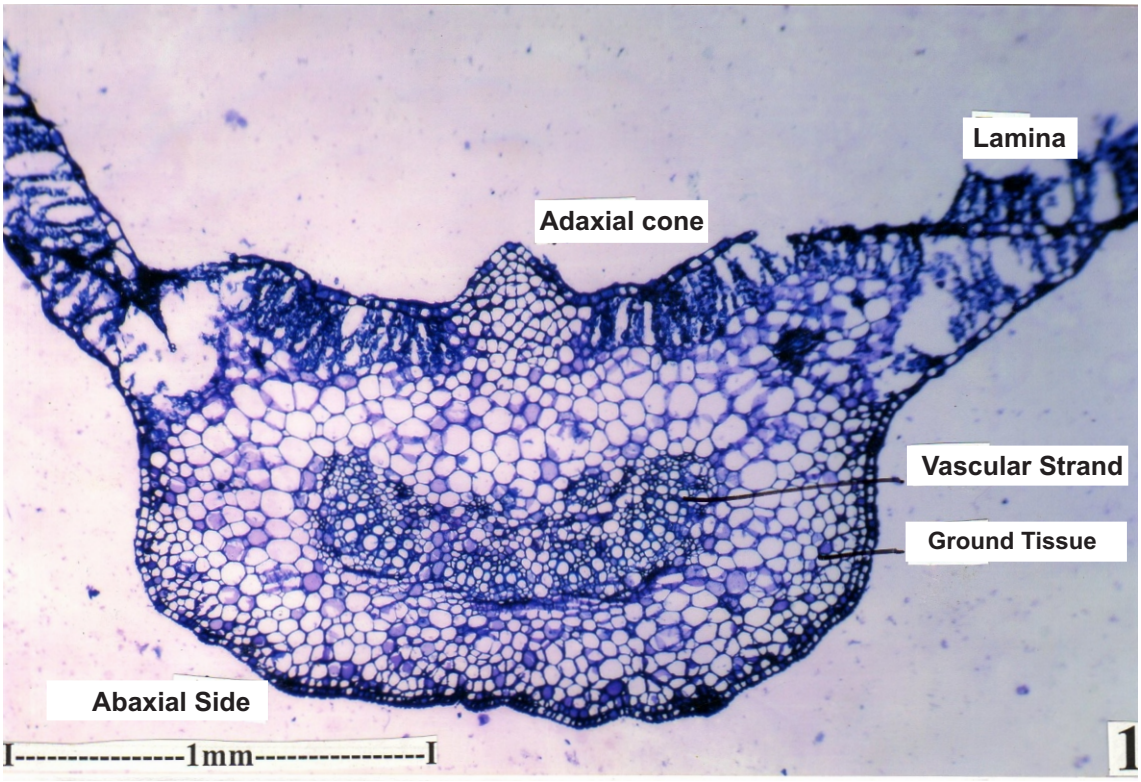


FIG. 11.2

T.S. of Lamina

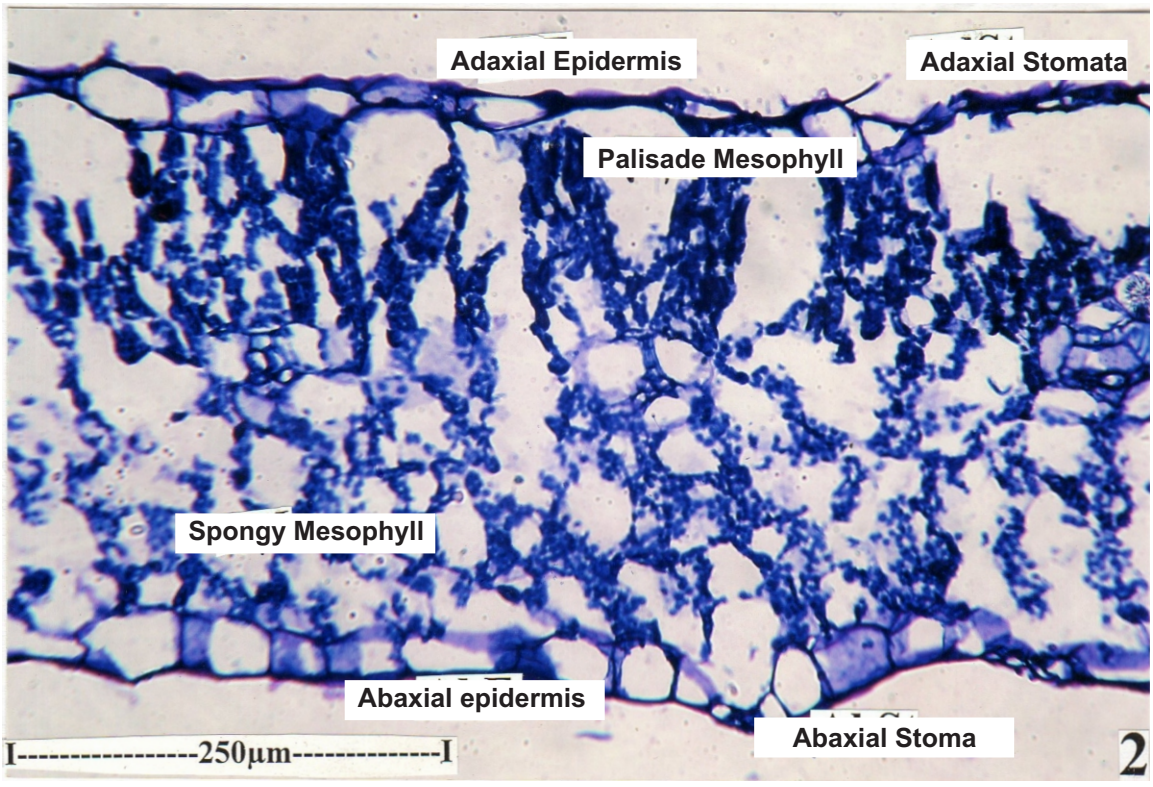


FIG. 12.1

Abaxial Phloem

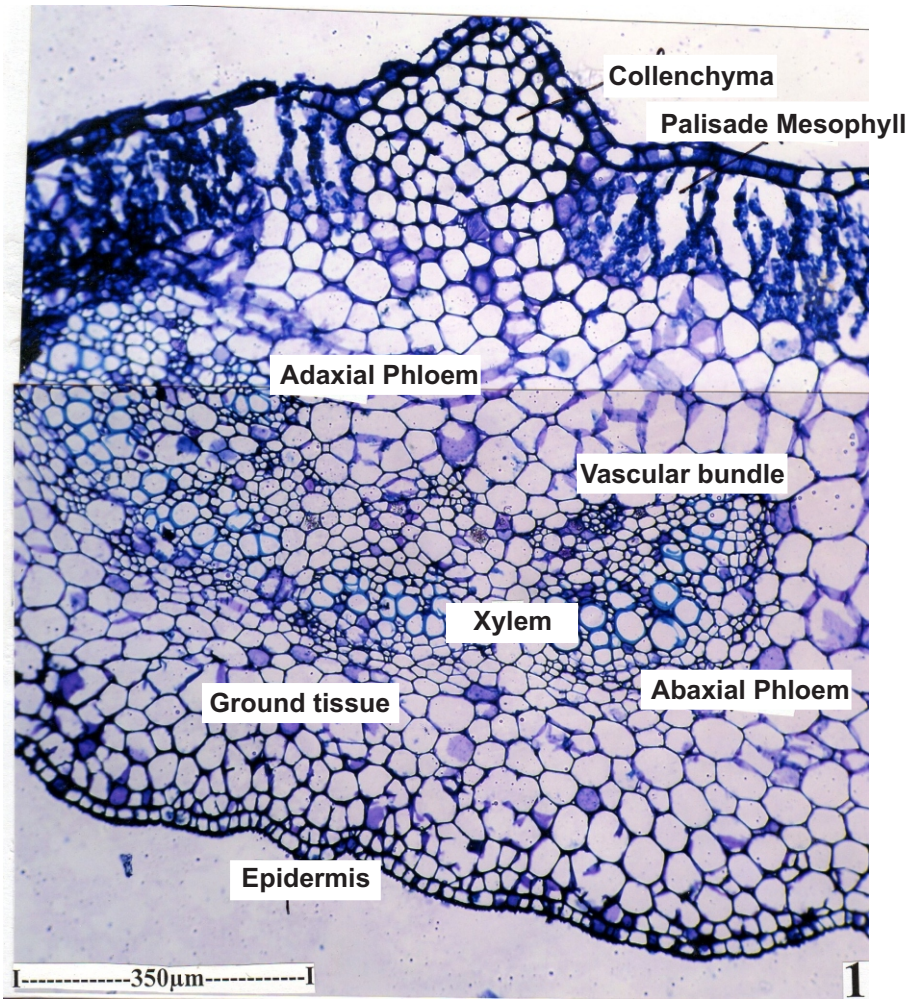


FIG. 12.2

Adaxial Phloem

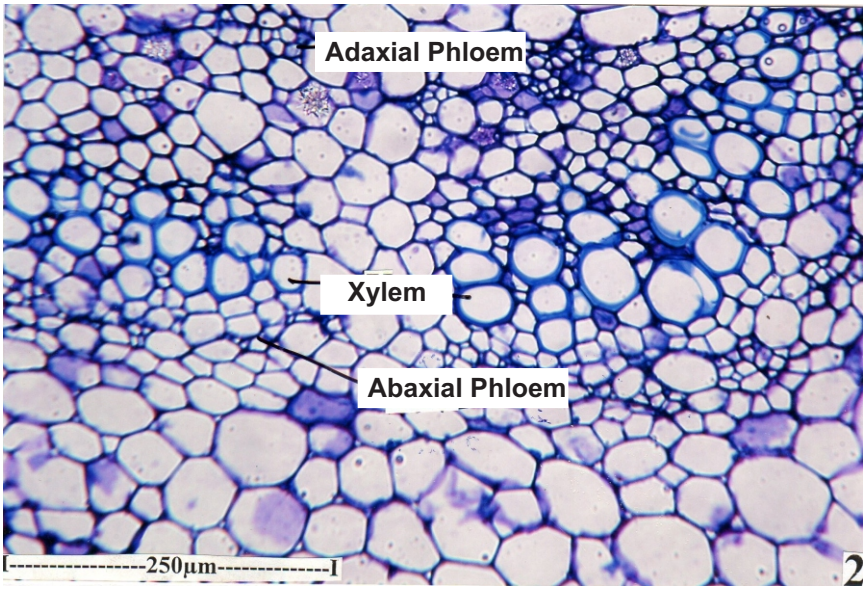


FIG. 13.1

Paradermal section of the abaxial epidermal layer showing the stomata

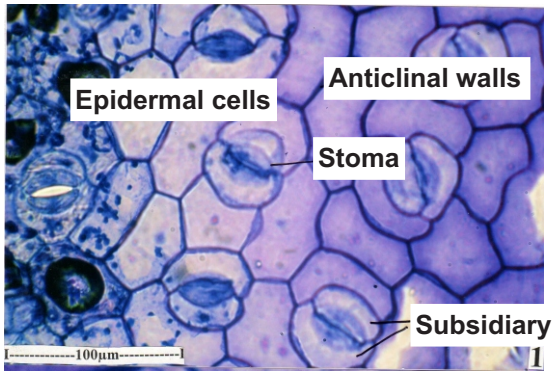


FIG. 13.2

Single paracytic stomata

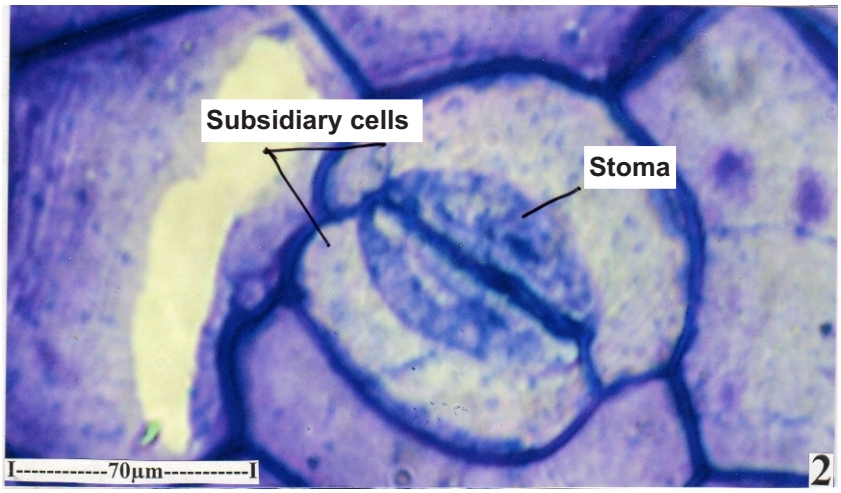


FIG. 13.3

Glandular trichome in surface view

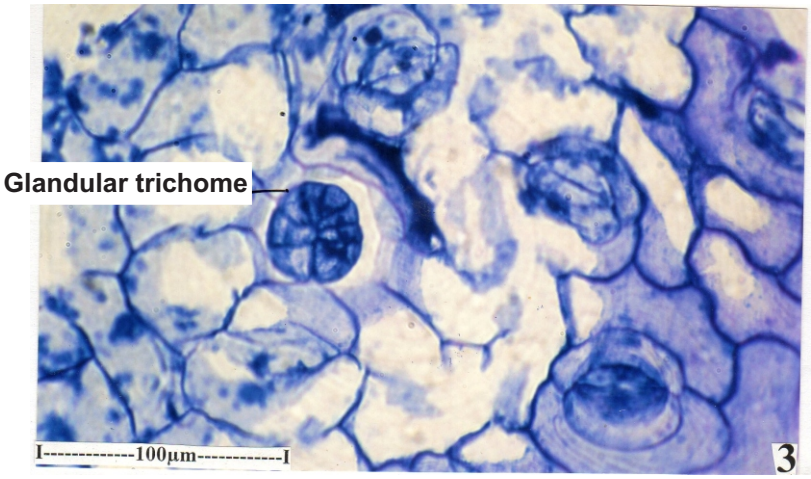


FIG. 14.1

Reticulate venation system of the lamina

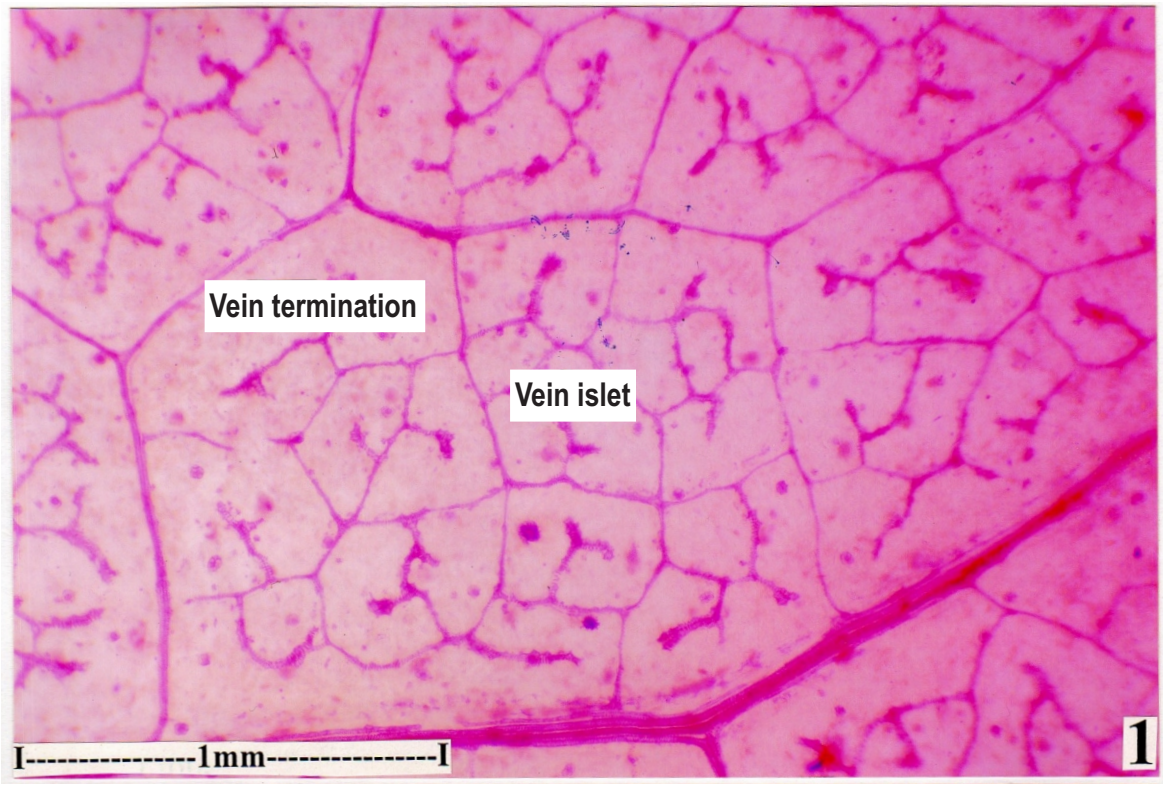
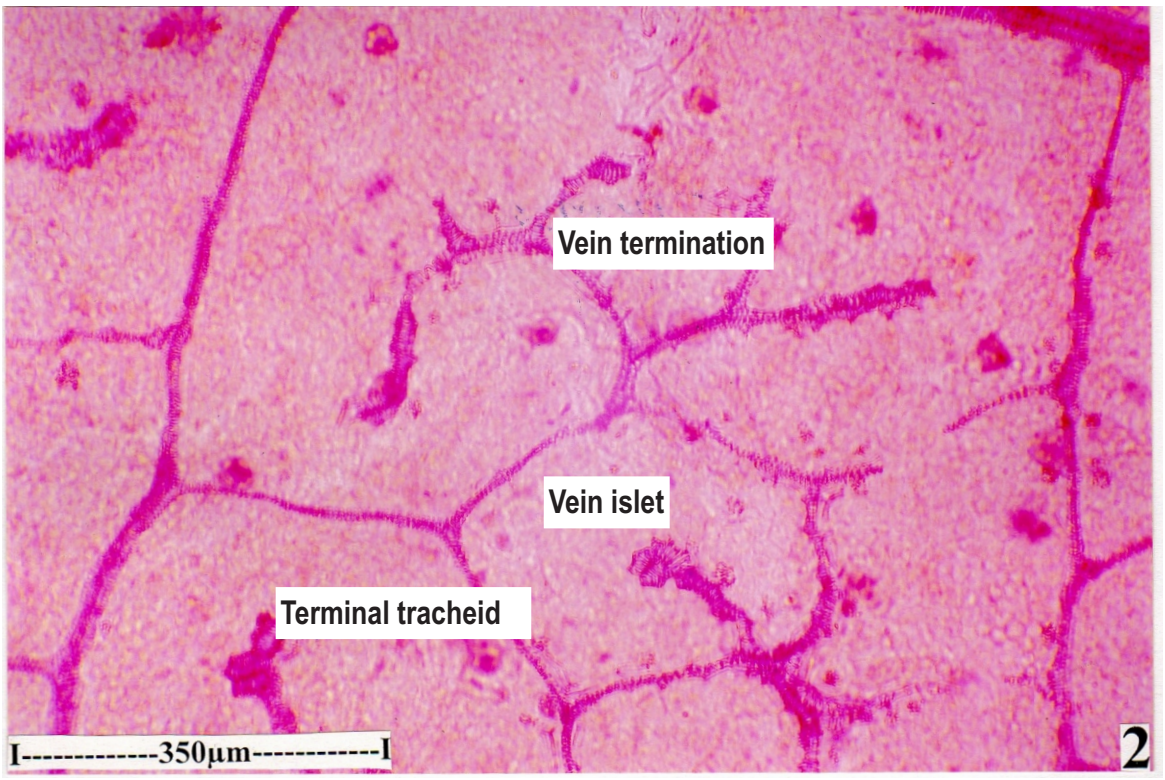


FIG. 14.2

Dendroid vein-termination with terminal tracheids



dendroid outline. At the ends of the vein termination, they are small clusters of terminal tracheids. **(fig 14.1, 14.2).**

Section – C Quantitative microscopy[69]

The results obtained for the determination of leaf constants like stomatal number, vein termination index and palisade ratio are presented in **table 1**

Table 1: Quantitative microscopical parameters of the leaf of *Ipomoea pes-caprae*

S. No.	Parameters	Values* obtained
1	Stomatal number	52.43
2	Stomatal index	14.65
3	Vein islet number	8.92
4	Vein termination number	11.28
5	Palisade ratio	4.32

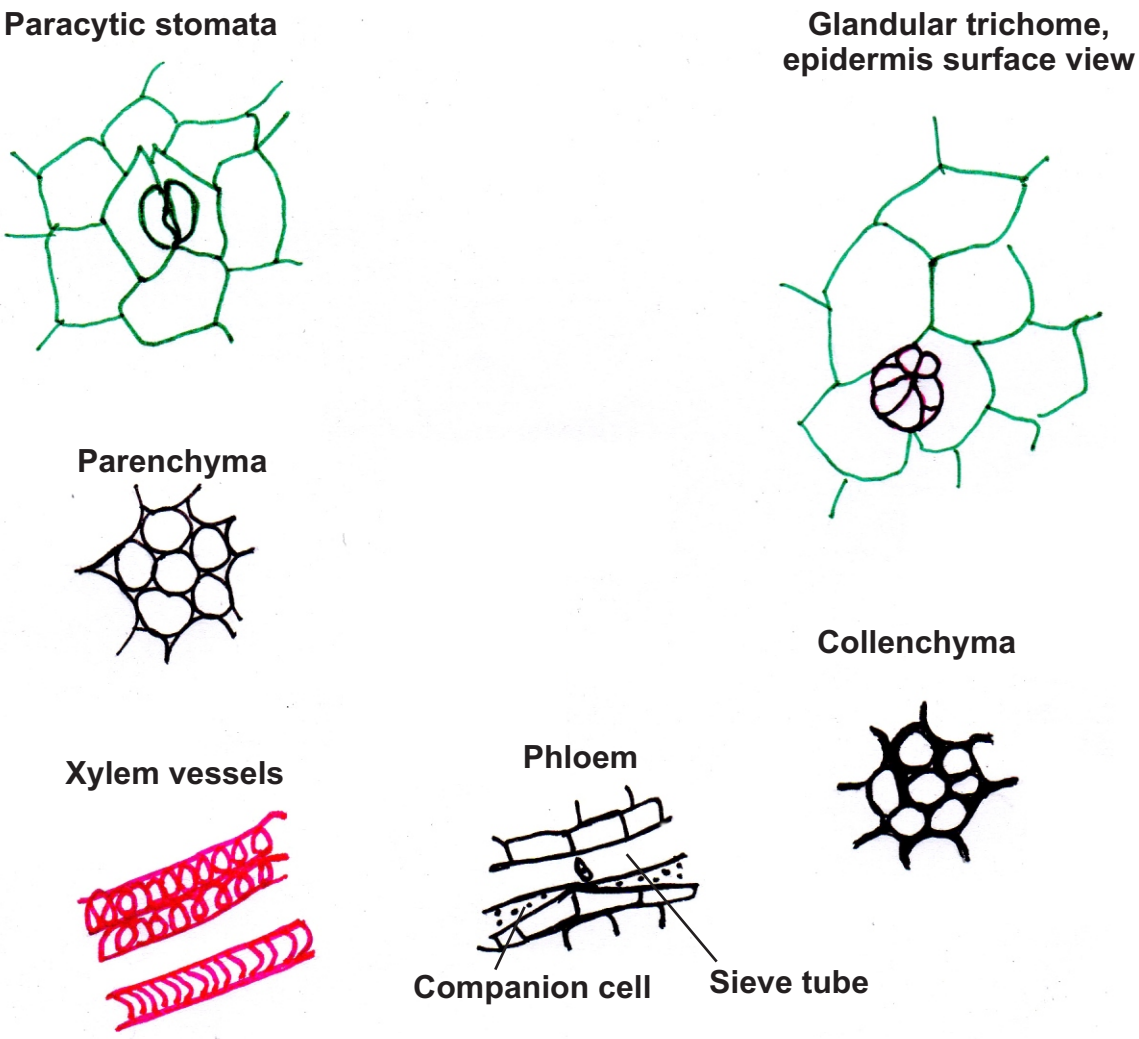
***mean of three readings** ± SEM

Section D- Powder microscopy and analysis

- Trichomes - glandular epidermal trichomes
- Stomata - Paracytic
- Collenchymas cells
- Parenchyma cells
- Xylem

FIG. 15

Powder microscopy of *Ipomoea pes-caprae*



Fluorescence analysis[79]

The organic molecules absorb light usually over a specific range of wave length and many of them emit such radiations. So if the powder is treated with different chemical reagents and seen in the UV chamber, different colours will be produced. The results of fluorescence analysis revealed the purity of this plant material [78]. The result is presented in **table-2.**

Table: 2 Fluorescence Analysis of extracts of *Ipomoea pes-caprae*

Extracts	**Consistency**	**Colour in Day Light**	**Colour under UV Lamp**	
			366 nm	**254nm**
Petroleum extract	Semisolid	Yellow	Orange	Yellow
Ether Extract	Semisolid	Greenish brown	Green	Greenish brown
Chloroform extract	Semisolid	Brownish green	Orange	Orange
Ethanol Extract	Semisolid	Light green	Green	Orange
Methanol extract	Semisolid	Green	Dark green	Orange
Aqueous extract	Semisolid	Brown	Brownish green	Dark green

Section E – Physical parameters

The result of the ash value,acid insoluble ash value and water soluble ash value presented in **table 3** .Total ash of the drug is inclusive as well as physiological ash. Physiological ash and non-physiological ash. Physiological ash is derived from the plant tissues. While non-physiological ash consists of residue of the extraneous matter(such as sand, soli etc),adhering to the herb itself. Many a time the crude drug

are admixed with various mineral substance like sand, soli, calciumoxalate, chalk powder or other drugs with different inorganic contents. For determining ash, the powdered drug is incinerate as to burn out all organic matter. Ash value is criterion to jude the identity or purity of crude drugs. Total ash usually consists of carbontes, oxides, phosphates, silicates and silica. Adhering dirt and sand may be determined by acid insoluble ash.

Table: 3 Analytical parameters of *Ipomoea pes-caprae*

S. No	Parameters	Values* expressed as %
1.	**Foreign organic matter**	0.01 ± 0.12
2.	**Moisture content**	11.85 ± 0.46
3**.**	**Ash values**	
	Total ash	8.01 ± 0.35
	Acid insoluble ash	4.32 ± 0.47
	Water soluble ash	4.2±0.90
	Water insoluble ash	7.8 ± 0.91

***mean of three readings** ± SEM

Extractive values

The result of the extractive values are presented in **table 4.** The extracts obtained by exhausting crude drug are indicative of approximate measure of their chemical constituents. Taking in to consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for determinations of extractives. The solvent used for extraction is in a position to dissolve appreciable quantities of substance desired. Highest extractive value was obtained for the hydro-ethanolic extractive of this plant (15.76%). It indicates that therapeutically viable active principles are predominantly present in hydro-ethanolic extract. Hence this extract was chosen for phyto chemical and pharmacological studies.

Table: 4 Analytical parameters – Extractive values of *Ipomoea pes-caprae*

S. No	Parameters*	Values* expressed as%
1.	**Extractive Values**	
	Petroleum extract	1.59± 0.48
	Chloroform extract	5.59± 0.04
	Ethanol extract	3.19 ±0.81
	Hydro ethanolic extract(Ethanol: Water, 70; 30)	15.76 ±0.71
	Methanol extract	13.57 ± 0.01
	Aqueous extract	9.67± 0.74
2.	**Foaming index**	less than 100
3.	**Swelling index**	**expressed as ml**
	Initial volume	3.6 ± 0.20
	Final volume	10.3 ± 0.97

***mean of three readings** ± SEM

PART II – PHYTOCHEMICAL EVALUATION

Section–A Preliminary phytochemical studies

Preliminary phytochemical study was performed for various extracts and powdered leaf material *of Ipomoea pes-caprae* and the results are tabulated in **table 5 and 6**.

Table: 5

Preliminary phytochemical screening for the leaf powder of *Ipomoea pes-caprae*

S.NO	TEST	RESULTS
1.	**TEST FOR STEROLS**	
	a. Salkowski's test	+
	b. Libermann- burchard's test	+
2.	**TEST FOR CARBOHYDRATES**	
	a. Molisch's test	+
	b. Fehling's test	+
	c. Benedict's test	+
3.	**TEST FOR PROTEINS**	
	a. Millon's test	+
	b. Biuret test	+
4.	**TEST FOR ALKALOIDS**	
	a. Mayer's reagent	+
	b. Dragendroff's reagent	+
	c. Hager's reagent	+
	d. Wagner's reagent	+
	e. Test for Purine group (Murexide test)	-
5.	**TEST FOR GLYCOSIDES**	
	a. Anthraquinone glycosides	+
	i) Borntrager's test	+
	ii) Modified Borntrager's test	+
	b. Cardiac glycosides	
	i) Keller Killiani test	-
	c. Cyanogenetic glycosides	-

6.	**TEST FOR SAPONINS**	+
7.	**TEST FOR TANNINS**	
	$Fecl_3$ test	+
8.	**TEST FOR FLAVONOIDS**	
	a. Shinoda test	+
	b. Alkali test	+
	c. Acid test	+
9.	**TEST FOR TERPENOIDS**	+
10.	**TEST FOR VOLATILE OILS**	-
11.	**TEST FOR MUCILAGE**	-

(+) indicates positive reaction (-) indicates negative reaction

Table: 6 Preliminary phytochemical screening for the various extracts of leaf powder of *Ipomoea pes-caprae*

S. No.	Chemical Test	Hexane extract	Petroleum ether extract	Chloroform	Methannolic extract	Ethanolic extract	Aqueous extract
1.	Terpenoids	+	+	+	+	+	-
2.	Flavonoids	-	-	-	+	+	+
3.	Phyto sterols	+	+	+	+	+	-
4.	Anthraquinone glycosides	-	-	-	+	+	+
5.	Cardiac Glycosides	-	-	-	+	+	-
6.	Sugars	-	-	-	+	+	+
7.	Alkaloids	-	-	-	+	+	-
8.	Quinones	-	-	-	-	-	-
9.	Phenols	-	-	-	+	+	+
10.	Tannins	-	-	-	+	+	+
11.	Saponins	-	-	-	+	+	+
12.	Proteins & free amino acids	-	-	-	+	+	+

(+) indicates positive reaction (-) indicates negative reaction

The rsults of the preliminary phytochemical studies showed the presence of terpenoids, tannins, phyto sterols, alkaloids, glycosides, flavonoids and saponins.

Section B – Quantitative estimation of phytoconstituents

Total Phenolic Content

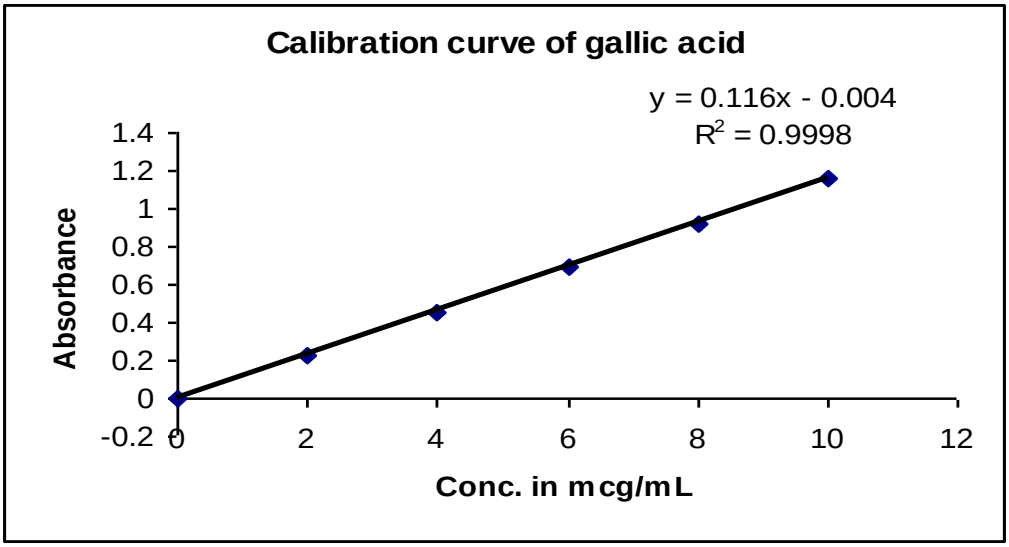
In the present study, total phenolic content present in the extract was estimated using modified Folin- ciocalteau method. Values are expressed as gallic acid equivalents and the results are tabulated in **table 7**. Total phenolic content for ethanolic extract of *Ipomoea pes-caprae* was found to be **22.27±0.19 mg/g.**The linear regression equation was found to be y=0.116x-0.004 while the correlation was found to be 0.9998.**(fig 16)**

Table 7 Total phenolic content in ethanolic extract of *Ipomoea pes-caprae*

S.NO	Con.c of gallic acid in µg/ml	Absorbance	Con.c of ethanolic extract in µg/ml	Absorance	Amount of total phenolic content in terms mg GAE/g of extract*
1	2	0.229 ± 0.010	50	0.138±0.001	23.97±0.25
2	4	0.452 ± 0.006	100	0.243±0.001	20.98±0.13
3	6	0.695 ± 0.005			
4	8	0.918 ± 0.031			
5	10	1.162 ± 0.028		**Average**	22.27±0.19

*** mean of three readings ±SEM**

Fig.16 : Calibration graph of gallic acid



Phenolics are the most widely distributed secondary metabolite in plant kingdom. These groups of compounds have much attention as potential natural antioxidants in terms of their ability to act as both efficient radical scavengers and metal chelator. It has been reported that the antioxidant activity of phenolic compounds are mainly due to their redox, hydrogen donating properties and singlet oxygen quenchers.[90]

Estimation of Flavonoid:

Flavonoid contents were determined by colorimetric method using $AlCl_3$. The flavones and flavonols react and form more stable complexes with Aluminium chloride. The amount of flavonoid was considered as the important index for evaluating the biological activity of drugs.

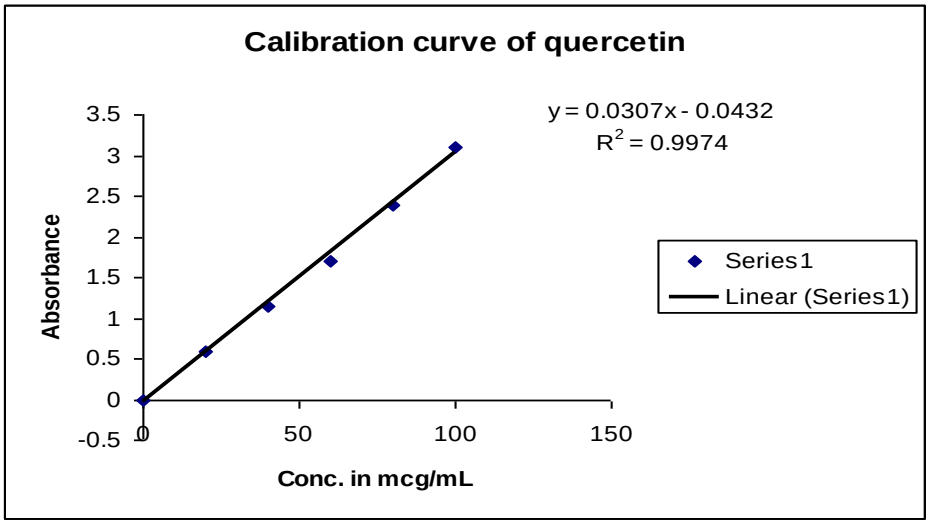
Total flavonoid content for ethanolic extract of leaves of *Ipomoea pes-capare* was found to be**217.1±0.37 mg/g** respectively. The linear regression equation was found to be y = 0.0307x - 0.0432 while the correlation was found to be 0.9974. **(fig 17)**

Table 8 Total flavonoid content of ethanolic extracts of *Ipomoea pescaprae*

S. No.	Conc. of quercetin in µg/Ml	Absorbance	Conc. of ethanolicextract in µg/Ml	Absorbance	Amt of total flavonoid content in terms of mg quercetin equivalent/ g of extract
1	20	0.589 ± 0.01	100	0.26±0.001	202.2±0.96
2	40	1.151 ± 0.04	200	0.66±0.002	232.1±10.79
3	60	1.710 ± 0.09			
4	80	2.390 ± 0.03			
5	100	3.112 ± 0.03		**Average**	217.1±0.37

***mean of three readings** ± SEM

Fig. 17: Calibration curve of Quercetin



Flavonoids are the most diverse group of polyphenols and are consist of a basic C_6-C_3-C_6 flavone skeleton. Six classes of flavonoids are widespread in higher plant, and include the chalcones, flavanones, flavandiols, flavones, anthocyanins, catechins, and condensed tannins. Its antioxidative activity is dependent on the chemical structure, such as the number of hydroxyl groups substituted on the B ring. Intake of flavonoids has been associated to decrease the incidences of cancer, heart disease.[89]

Total Tannin Content

Total tannin determination is carried out by spectrophotometry after oxidation of the analyte with the Folin–Denis reagent in alkaline medium. This method is based on a redox reaction and other reducing agents in the samples.

Total tannin content for ethanolic extract of leaves of *Ipomoea pes-caprae* was found to be **157.25±3.99 mg/g** respectively.

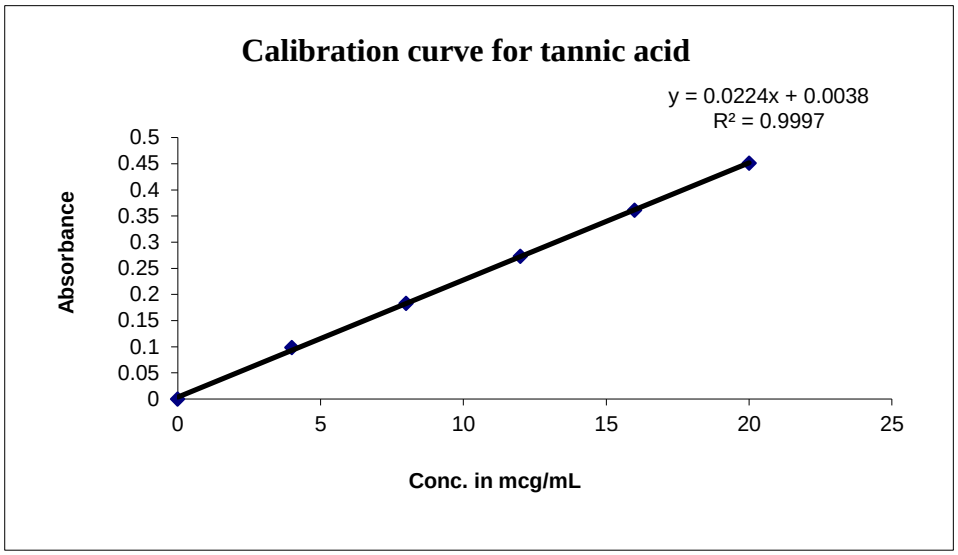
Table 9 Total tannin content in ethanolic extracts of *Ipomoea pes-caprae*

S. No.	Conc. of Tannic acid in µg/Ml	Absorbance	Conc. of ethanolic extract in µg/ml	*Absorbance	*Amt of total Tannin content in terms mg tannic acid equivalent/ g of extract
1	20	0.589 ± 0.01	10	0.03±0.000	124.3±0.46
2	40	1.151 ± 0.04	20	0.05±0.0005	114.2±0.28
3	60	1.710 ± 0.09	40	0.07±0.0005	75.81±0.07
4	80	2.390 ± 0.03			
5	100	3.112 ± 0.03			
				Average	157.25±0.99

***mean of three readings ± SEM**

The linear regression equation was found to be y = 0.022x + 0.003 while the correlation was found to be **table 9**. The amount of tannin content present in the ethanolic extract of *Ipomoea pes-caprae* was found to be **157.25±0.99mg/g** respectively.**(fig 18)**

Fig 18: Calibration curve for Tannic acid



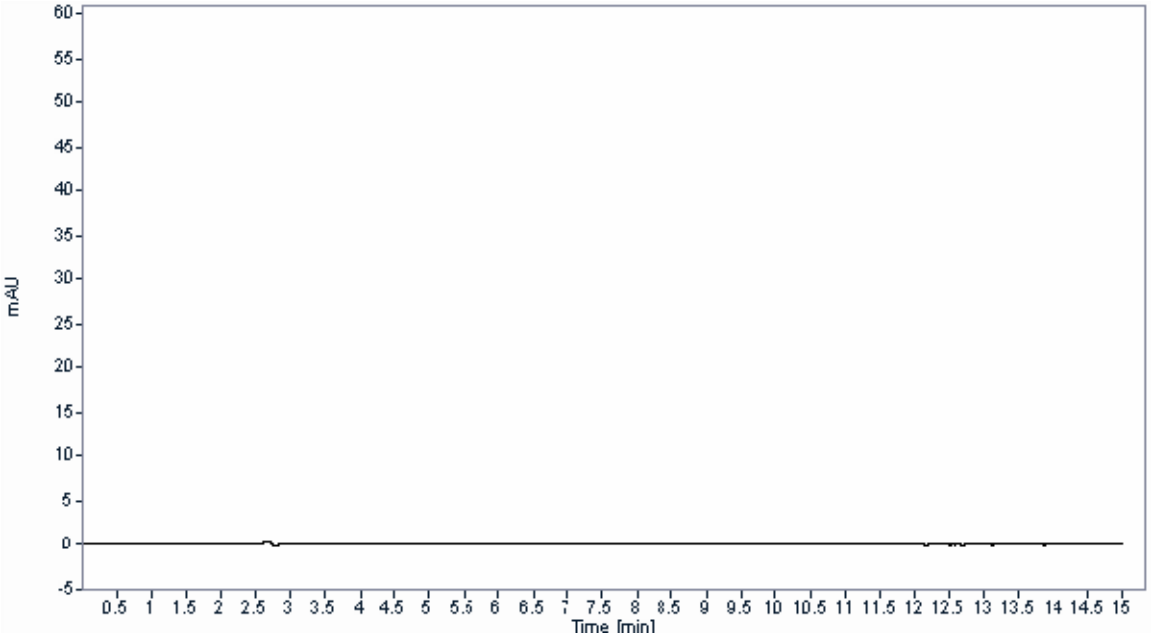
It is known that plant tannin can be precipitated by many chemical reagents and these precipitation techniques have become the tool for the estimation. Tannins are antioxidants phytoconstituents.

Section C – High performance liquid chromatography

Analysis and quantification of kaempferol in *Ipomoea pes-caprae* extract by HPLC

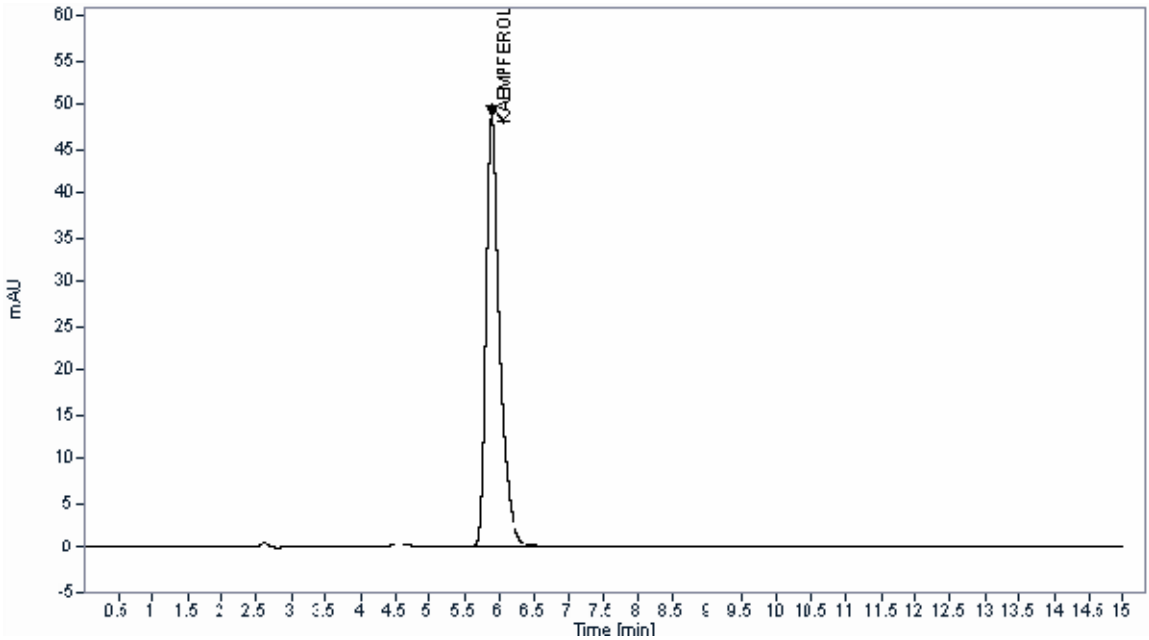
The results of the HPLC chromatogram of standard kaempferol and IPC extract are presented in (**fig 19-25)** and **table 10**. HPLC chromatogram revealed that the retention time (t_R) of standard kaempferol was found to be **5.88 mins** (t_R). The retention of a peak appeared in the chromatogram of IPC extract was found to be **5.87 mins** (t_R) and it indicates the presence of kaempferol in the IPC extract. The percentage content of kaempferol present in the air dried leaf plant material of IPC was calculated as **0.034 %.** So for kaempferol has not been reported in this plant, but it has been reported in other species of this plant.[92]

Fig-19 HPLC Chromatogram of kaempferol (Dil. Methanol)



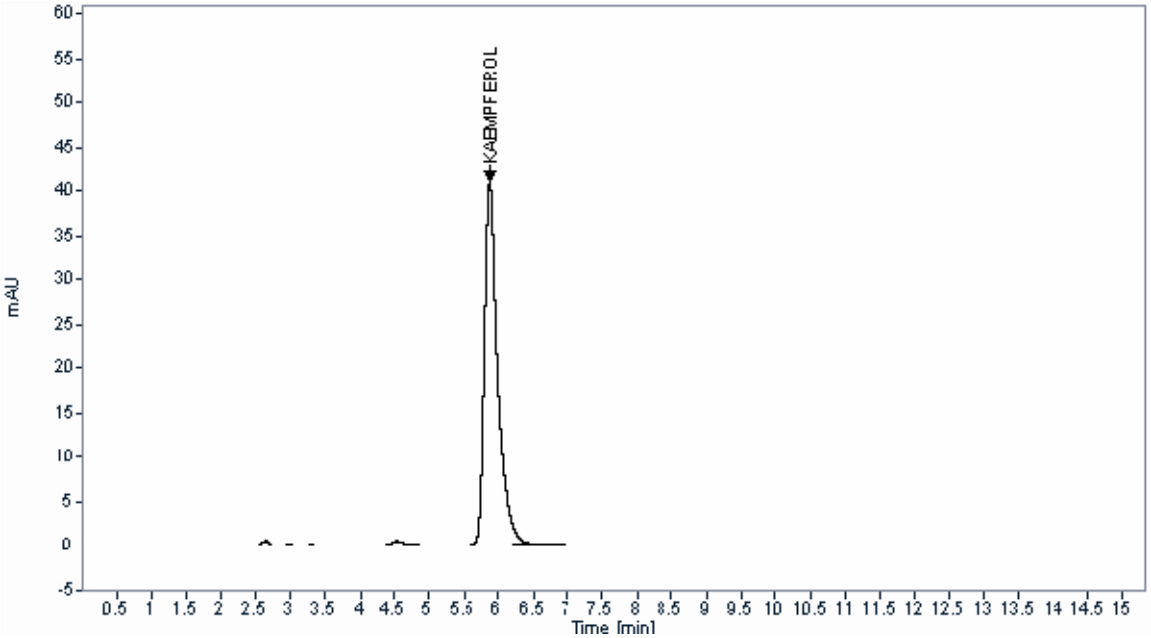
Name	RT[min]	Area	Area %	TP	TF
	Sum				

Fig- 20 HPLC Chromatogram of kaempferol (19.04 µg/mL)



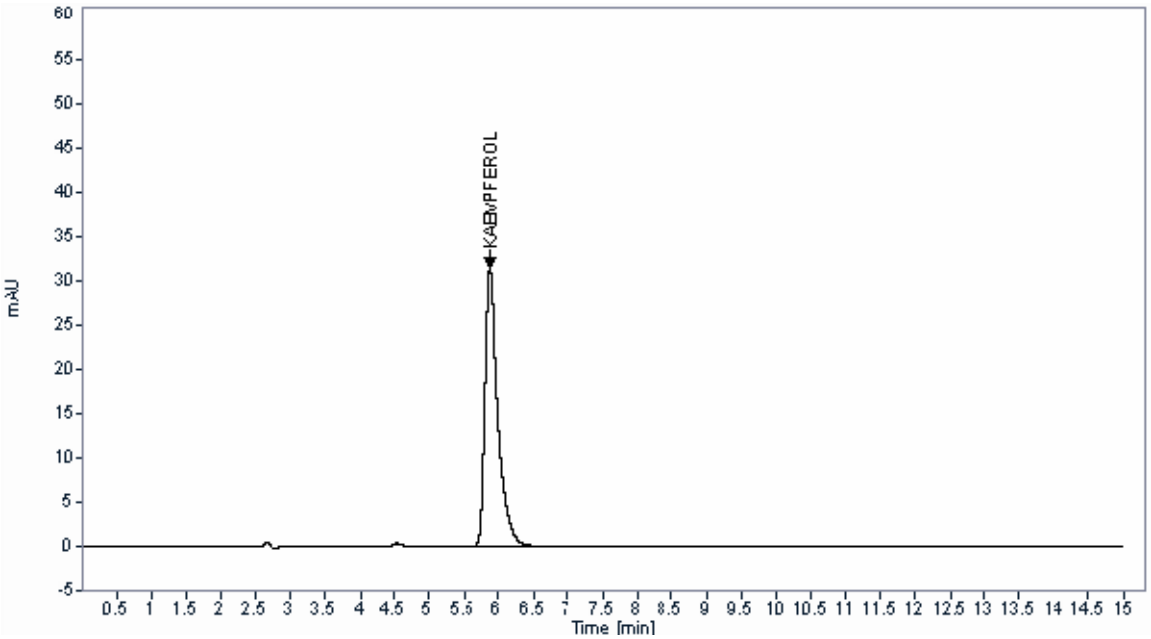
Name	RT[min]	Area	Area %	TP	TF
KAEMPFEROL	5.88	665.26	100.00	4967	1.49
	Sum	665.26	100.00		

Fig- 21 HPLC Chromatogram of kaempferol (15.23 µg/mL)



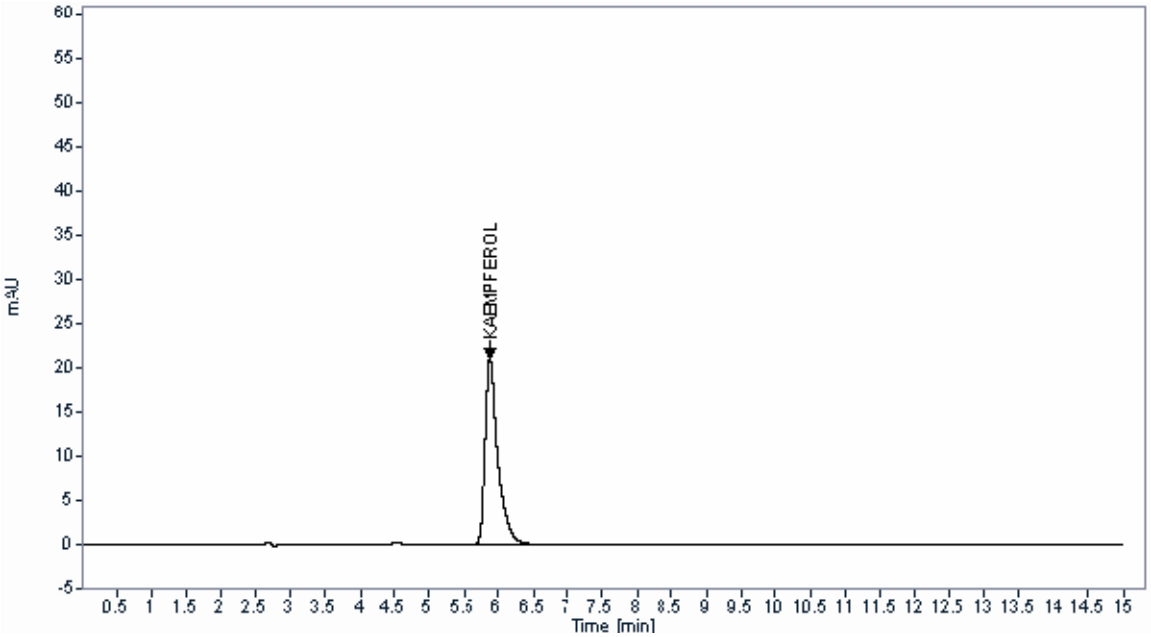
Name	RT[min]	Area	Area %	TP	TF
KAEMPFEROL	5.88	533.50	100.00	5544	1.58
	Sum	533.50	100.00		

Fig-22 HPLC Chromatogram of kaempferol (11.42 µg/mL)



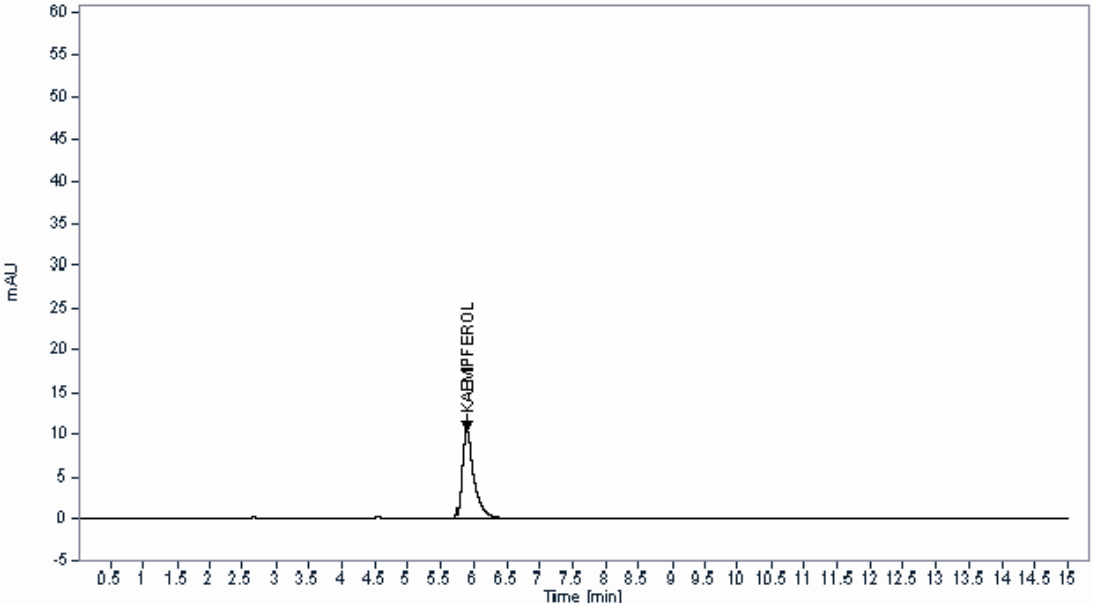
Name	RT[min]	Area	Area %	TP	TF
KAEMPFEROL	5.88	397.52	100.00	6028	1.63
	Sum	397.52	100.00		

Fig -23 HPLC Chromatogram of kaempeforl (7.61mg/mL)



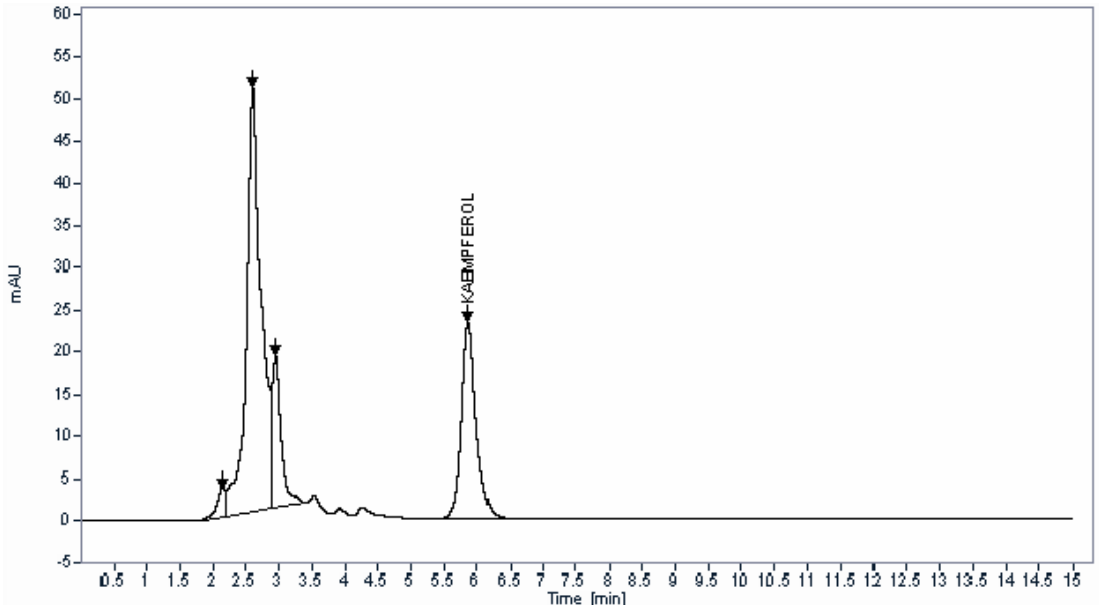
Name	RT[min]	Area	Area %	TP	TF
KAEMPFEROL	5.88	263.70	100.00	6230	1.67
	Sum	263.70	100.00		

Fig -24 HPLC Chromatogram of kaempferol (3.81 mg/mL)



Name	RT[min]	Area	Area %	TP	TF
KAEMPFEROL	5.88	127.99	100.00	6422	1.65
	Sum	127.99	100.00		

Fig -25 HPLC Chromatogram of IPC extract



Name	RT[min]	Area	Area %	TP	TF
	2.13	30.07	2.21	290	0.66
	2.60	793.88	58.23	1079	0.85
	2.96	176.75	12.96	1250	2.94
KAEMPFEROL	5.87	362.76	26.61	3770	1.21
	Sum	1363.46	100.00		

PART – III PHARMACOLOGICAL EVALUATION

Section A - *In vitro* ant oxidant activity

Antioxidants obtained from plant are of greater benefit in comparison to synthetic one. It protects against free radicals and they are therefore essential in obtaining and preserving good health.

Method 1 :Hydrogen peroxide scavenging activity assay:

The ethanolic extracts of *Ipomoea pes-caprae* was also evaluated for hydrogen peroxide scavenging activity assay. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with $Fe^{2+,}$ and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The decomposition of H_2O_2 by *I. pes-caprae* may at least partly result from its antioxidant and free radical scavenging activity.

$$H_2O_2 + Cu+ \text{ or } Fe^{2+} \rightarrow Cu^{2+} \text{ or } Fe^{3+} + OH + \cdot OH-$$

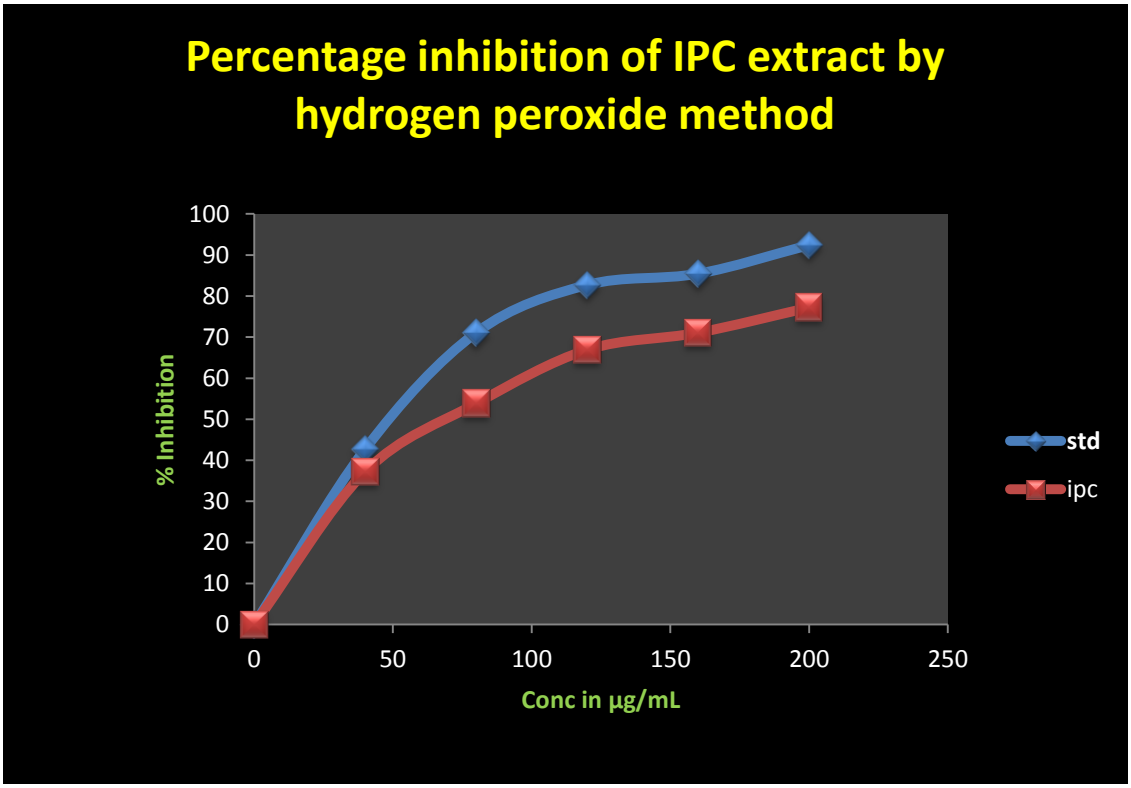
The scavenging of hydrogen peroxide by ethanolic extracts of *Ipomoea pes-caprae* may be due to the presence of phenolic compounds. $IC_{50}$and percentage inhibition of hydrogen peroxide scavenging activity of ethanolic extract was tabulated in **table 10**.**(fig 26)**

Table-10 Percentage inhibition of EEIPC by hydrogen peroxide method

S. No.	Conc. in µg/mL	Percentage inhibition by standard ascorbic acid	Percentage inhibition by EEIPC
1	40	42.72±0.68	37.2±0.34
2	80	71.08± 0.89	53.83±1.14
3	120	82.7± 0.96	66.9±0.95
4	160	85.45± 0.86	71.0±0.83
5	200	92.42± 0.65	77.2±0.82
	IC_{50}	**71.32µg/mL**	**97.28µg/Ml**

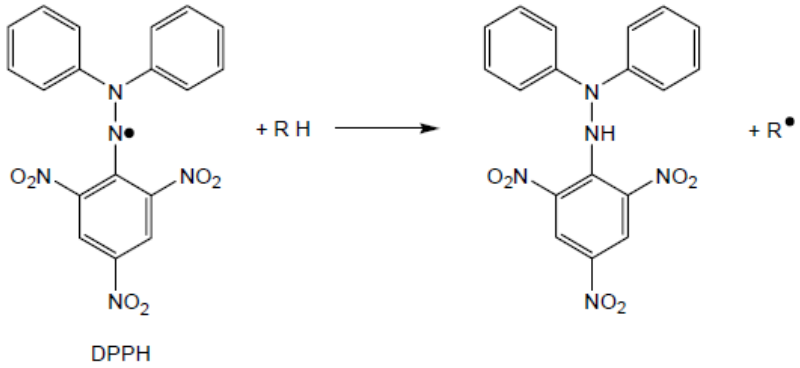
*** mean of three readings± SEM**

FIG 26; Determination of scavenging activity of IPC extract against hydrogen peroxide



Method 2 :DPPH radical scavenging activity:

The scavenging of the DPPH radical by hydrogen donating antioxidant is characterized by a rapid decline in the absorbance at 517 nm. The rapid reaction between antioxidants and DPPH occurs with the transfer of the most labile H atoms to the radical, while the subsequent slow step depends on the residual H-donating capacity of antioxidant degradation products. The antioxidants react with DPPH and convert it to 1, 1-diphenyl-2-picryl hydrazine with decolouration (from deep violet to light yellow).



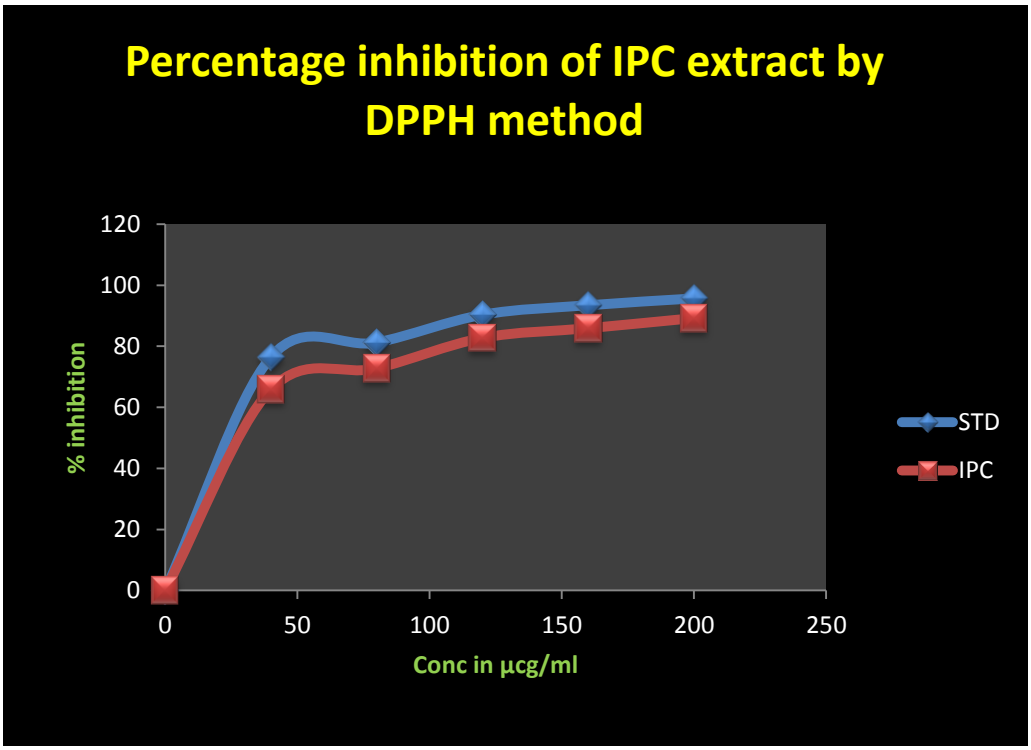
Ascorbic acid is used as standard. The radical scavenging activity of ethanolic extract *Ipomoea pes-caprae* is tabulated in **table 11.**

Table 11: DPPH radical scavenging assay of IPC extract

S. No.	Conc. in µg/mL	Percentage inhibition by ascorbic acid	Percentage inhibition by *Extract*
1	40	76.44±1.68	65.86±0.55
2	80	81.22±1.22	72.77±0.69
3	120	90.33±2.38	82.75±0.32
4	160	93.45±1.47	85.92±0.87
5	200	95.68±2.02	89.24±0.63
	IC_{50}	**40.67µg/mL**	**56.52µg/mL**

***mean of three readings*±SEM**

FIG 27 :Determination of scavening activity of IPC extract against DPPH method



Method 3: The total antioxidant capacity:

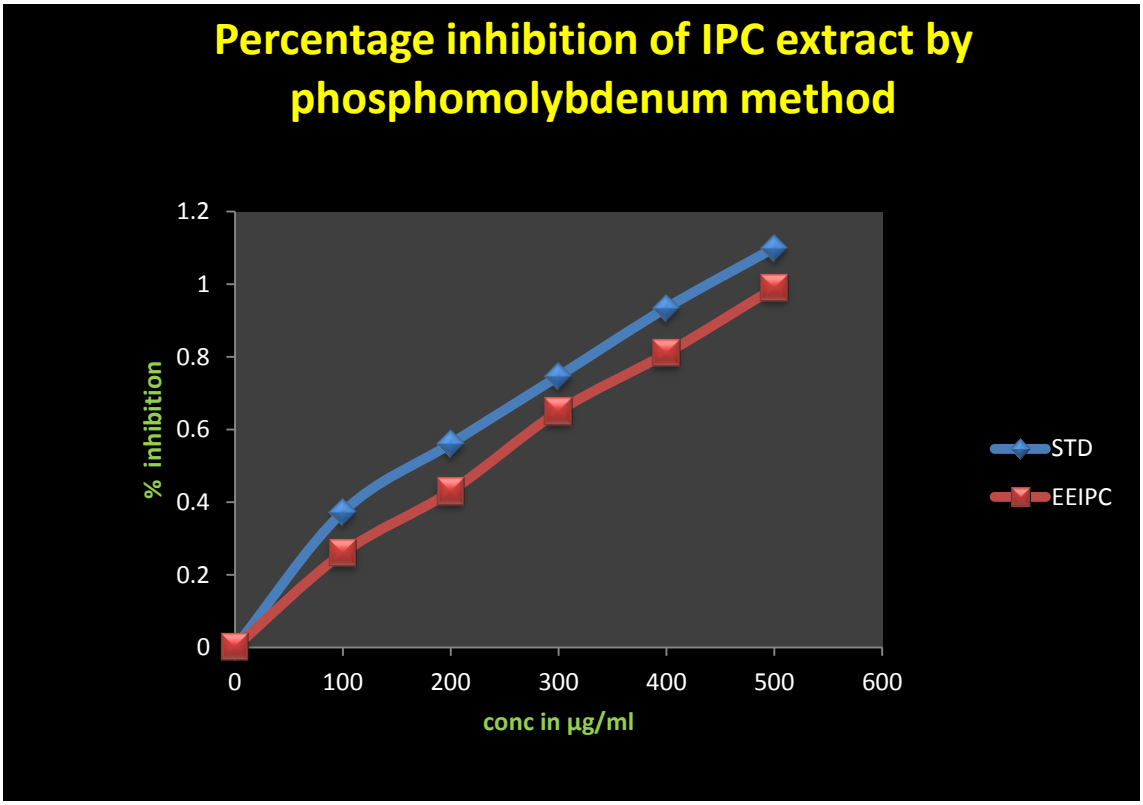
Free radical scavenging of phenolic compounds is an important property underlying their various biological and pharmacological activities. The Phosphomolybdenum assay is based on the reduction of Mo (VI) to Mo (V) by antioxidant compounds and a formation of green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The anti oxidant assay of EEIP due to presence of phenolic compounds and flavonols. Total antioxidant capacity of EEIP was presented in **table 12**.**(fig 28)**

Table 12: Percentage inhibition of IPC extract by Phosphomolybdenum method

S. No.	Conc. in µg/mL	Percentage inhibition by standard ascorbic acid	Percentage inhibition by extract
1	100	0.373±0.06	0.26±0.03
2	200	0.561±0.01	0.43±0.03
3	300	0.747±0.04	0.65±0.01
4	400	0.935±.007	0.81±0.001
5	500	1.10±0.09	0.99±0.05

***mean of three readings ± SEM**

FIG 28 :Total antioxidant assay of IPC extract



Method 4 : Reducing power assay

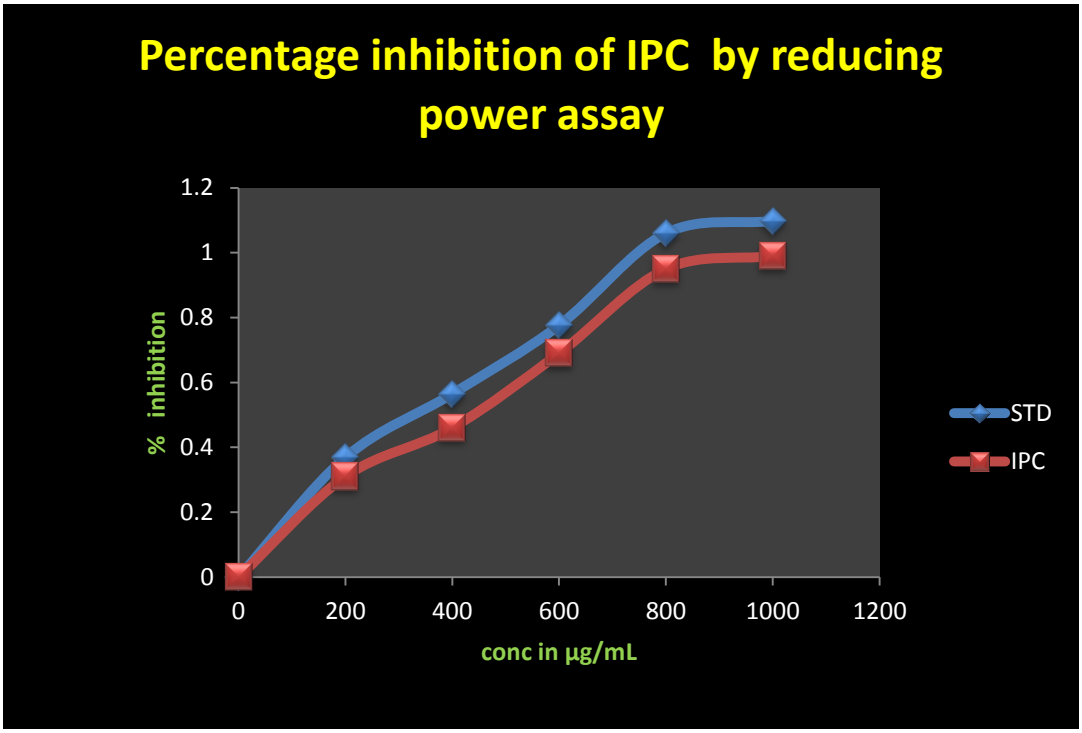
The results obtained for the free radical scavenging activity against Reducing power assay are presented in **Table 15. (fig 29)**

Table 13 : Reducing power assay of IPC extract

S. No.	Conc. in µg/Ml	Percentage inhibition by standard ascorbic acid	Percentage inhibition by extract
1	100	0.369±0.03	0.31±0.009
2	200	0.565±0.01	0.46±0.02
3	300	0.776±0.04	0.69±0.04
4	400	1.06±0.01	0.95±0.02
5	500	1.098±0.02	0.99±0.05

***mean of three readings ± SEM**

FIG 29; Reducing power assay of IPC extract

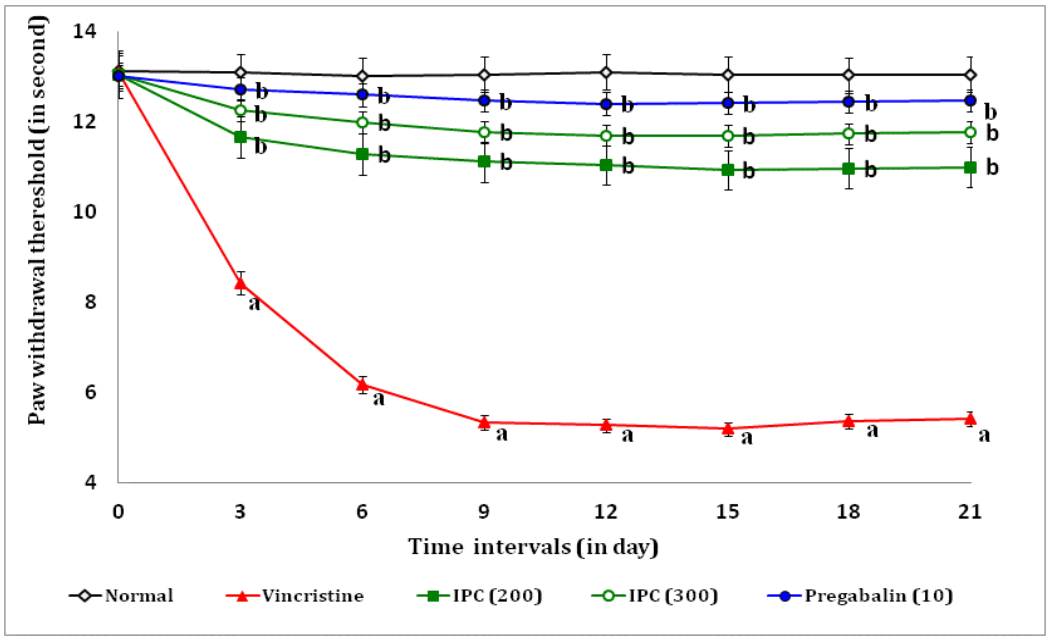


Section B- Effect of ethanolic extracts of IPC extract on vincristine induced neuropathic pain in rats

Effect of IPC extract on paw heat hyperalgesic test

Administration of vincristine caused significant development noxious thermal hyperalgesia noted by decrease in hind paw withdrawal threshold after 3rd day of vincristine administration when compared to normal control group. Vincristine induced, decrease in nociceptive threshold for thermal hyperalgesia was improved by the administration of IPC (200 and 300 mg/kg, *p.o.)* in a dose dependent manner ($p<0.05$). Treatment of pregabalin also produced similar effects. Normal control group of animals did not show any significant effect on heat hyperalgesic test.**(Fig 30)**

FIG. 30 Effect of *IPC extract* on paw heat hyperalgesic test.



Digits in parenthesis indicate dose in mg/kg.

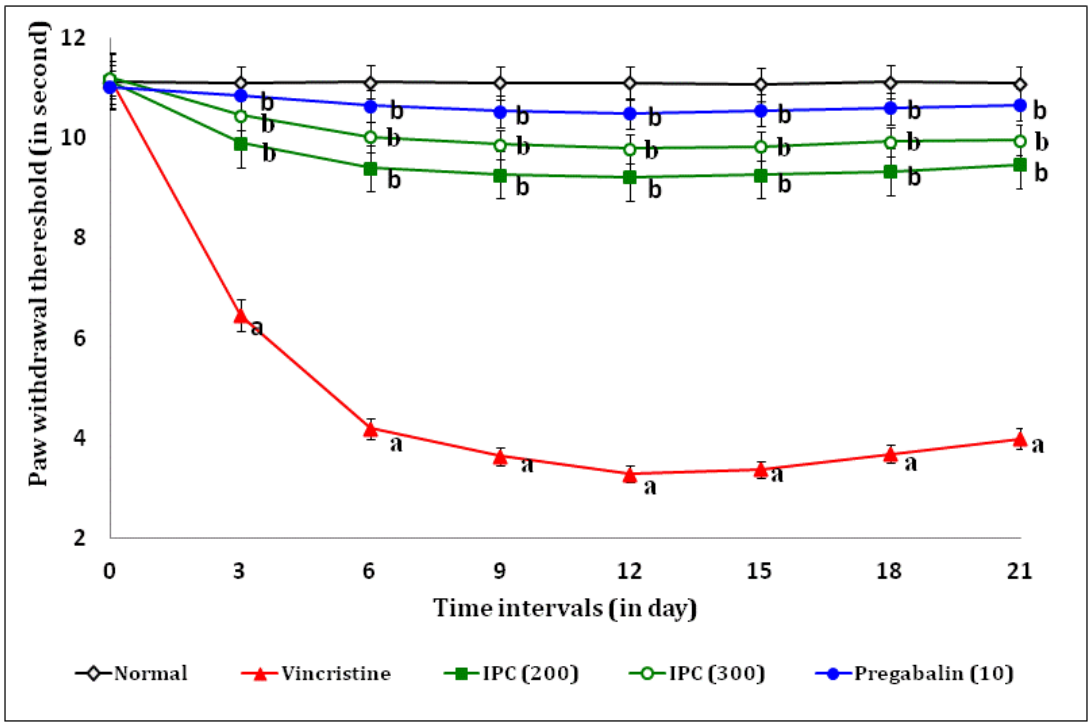
IPC

Data were expressed as mean ± SEM, n=6 rats per group.[a]$p<0.05$ vs normal control group.[b]$p<0.05$ vs vincristine control group.

Effect of IPC on paw cold allodynia test

Vincristine teratment lead to the development of paw cold-allodynia indicateted by decrease in nociceptive threshold when compared to normal control group of animals.Treatment of IPC at 200 and 300 mg/kg,*p.o.* improved the nociceptive threshold in a dose dependent manner ($p< 0.05$).Similar result was obtained with pregabalin treatment. Normal control animals did not show any effect on paw cold allodynia test. **(fig 31)**

FIG. 31 Effect of *IPC* extract on paw cold allodynia test.



Digits in *parenthesis* indicate dose in mg/kg.

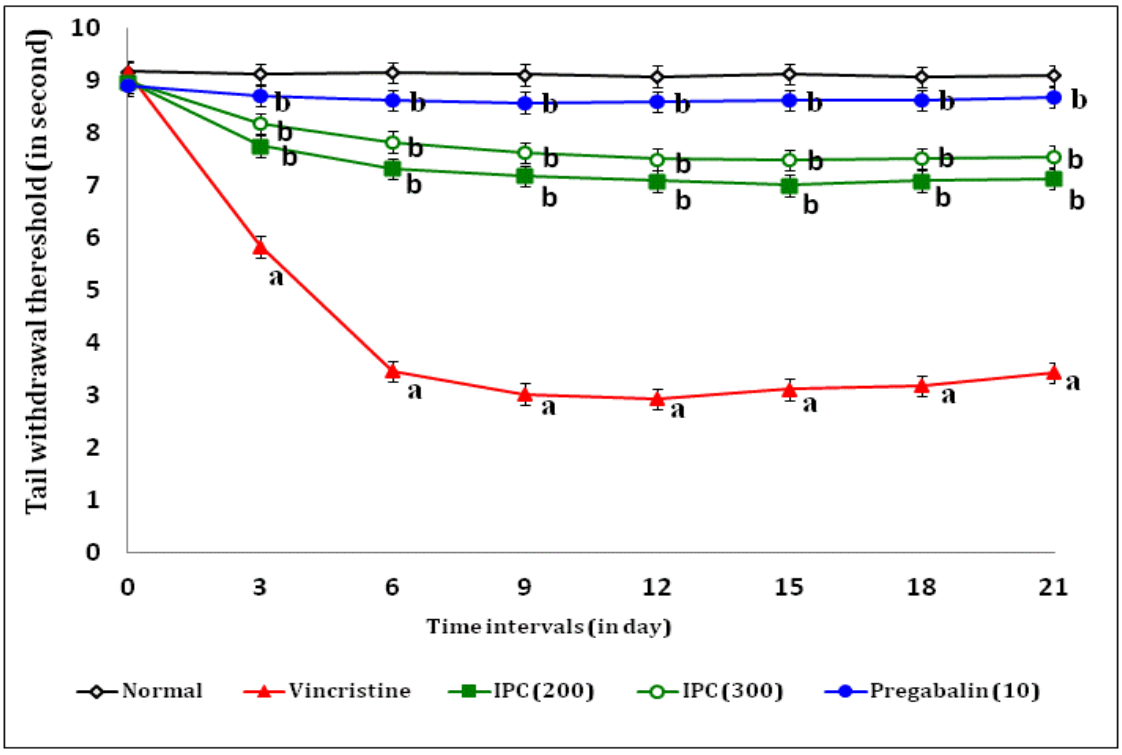
IPC

Data were expressed as mean ± SEM, n=6 rats per group.[a]$p<0.05$ vs normal control group.[b]$p<0.05$ vs vincristine control group.

Effect of IPC on tail immersion test

Vincristine administration caused significat development of noxious thermal hyperalgesia noted by decrease in a tail withdrawal threshold, after 3rd day of vincristine administration when compared to normal control animals. Vincristine induced, decrease in nociceptive threshold for thermal hyperalgesia was improved by administration of IPC at 200 and 300 mg/kg,*p.o.* in adose dependent manner ($p < 0.05$). Treatment of pregabalin also produced similar effects. Normal control animals did not show any effect on tail immersion test. **(fig 32**)

FIG.32 Effect of *IPC* extract on tail heat hyperalgesia



Digits in parenthesis indicate dose in mg/kg.

IPC

Data were expressed as mean ± SEM, n=6 rats per group.

[a]*p*<0.05 vs normal control.

Effect of IPC on nerve tissue biomakers changes

Vincristine administsrtion caused in asignificant increase in the levels of biomarkers i.e., total calcium and TBARS and decrease in the level of GSH. Treatment of IPC extract at 200 and 300mg/kg, significantly attenuated the elevated total calcium and TBARS and increased the level of GSH in a dose dependent manner when compared to normal control group of rats ($p< 0.05$).Similar effect were seen with pregabalin administration.The results are persented in **table 14.**

Table 14 Effect of *IPC* extract on tissue biomarker changes

Groups	**Total Protein** (mg /g of tissue)	**TBARS** (nmol/mg of protein)	**GSH** (µg/mg of protein)	**Total calcium** (ppm/mg of protein)
Normal	0.56 ± 0.22	2.97 ± 0.46	83.19 ± 1.97	3.04 ± 0.19
Vincristine	0.62 ± 0.19	5.05 ± 0.27[a]	42.74 ± 3.64 [a]	23.18± 0.51 [a]
Pregabalin (10)	0.67 ± 0.15	3.07 ± 0.37 [b]	79.63 ± 2.05 [b]	6.23± 0.34[b]
IPC (200)	0.59 ± 0.27	3.98 ± 0.24[b]	61.73 ± 2.74 [b]	12.63 ± 0.28[b]
IPC (300)	0.64 ± 0.14	3.43 ± 0.21 [b]	73.74 ± 2.84[b]	9.94 ± 0.52 [b]

Digits in parenthesis indicate dose in mg/kg.

TBARS, thiobarbituric reactive substances; GSH, reduced glutathione;

IPC

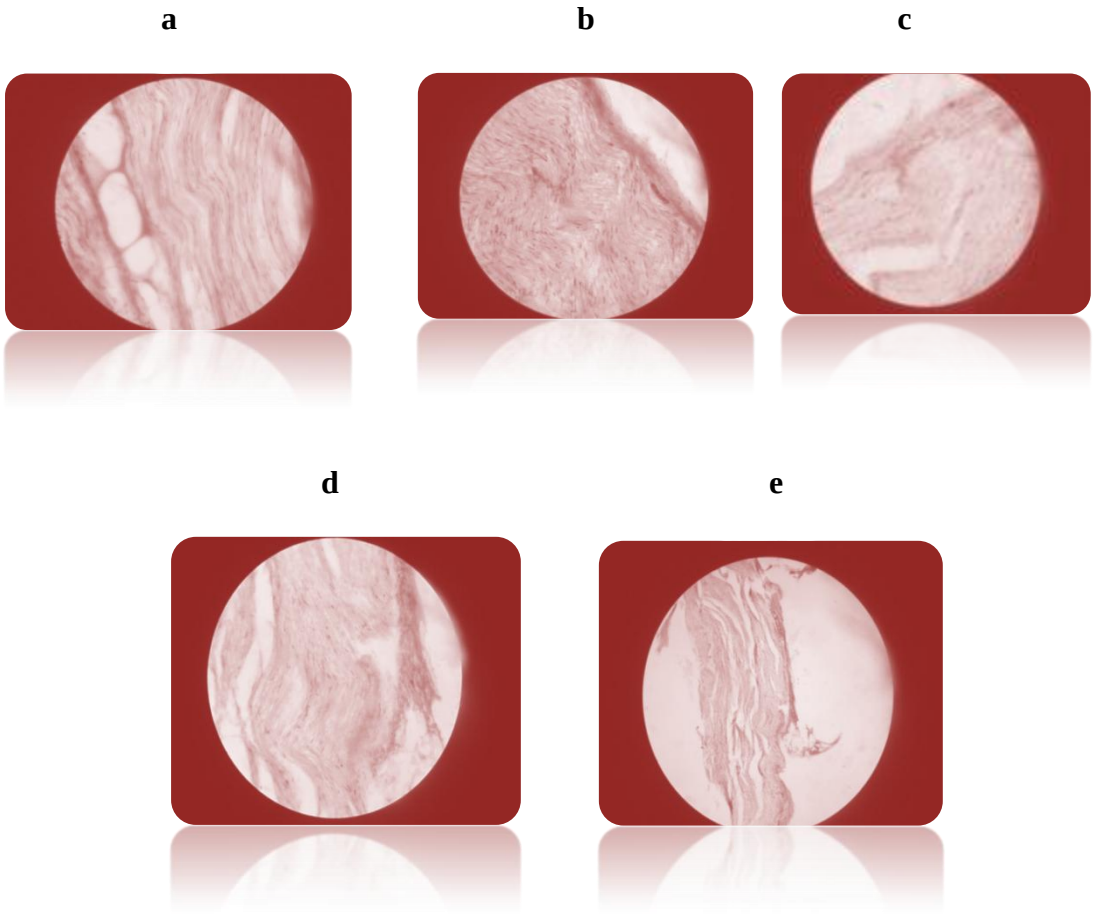
.Data were expressed as mean ± SEM, n= 6 rats per group. [a]p<0.05 vs normal control group.[b]p<0.05 vs vincristine control group.

group.[b]p<0.05 vs vincristine control

Histopathological studies

The sciatic nerve of vincristine group showed nerve derangement and axonal swelling. Treatment with hydroalcoholic extract of IPC (200 and 300mg/kg, *p.o.*) significantly attenuated vincristine induced histopathological alterations.Similar effects were observed with pregabalin treatment.**(fig 33)**

FIG 33 ; Effect of vincristine induced histopathological changes.



Parts a-e show transverse section of sciatic nerve of normal, vincristine, IPC (200 and 300 mg/kg) and pregabalin treated groups.

DISCUSSION[117-119]

In this present study IPC extracts ameliorated vincristine induced behavioral (thermal hyperalgesia and cold chemical allodynia) and biochemical (TBARS,GSH and Total calcium) changes. After vincristine treatment the behavioral alterations started from day 3 and the maximal nociceptive threshold was observed on the 9th day. These observations are in line with reports from other laboratories. Vincristine has been widely used for the management of various cancer disorders including Hodgkin's disease. However, its clinical application has been limited due to unavoidable painful neuropathy. It possess high binding affinity towards β-tubulin (neuronal cytoskeleton protein). Subsequently it has been documented to cause disruption of microtubule polymerization eventually leading to neurotoxicity as well as cancer preventive (Swain and Arezzo, 2008). Vincristine treatment in our study produced an increase in the levels of calcium, TBARS and decrease in the level of GSH. However increase in ca^{2+} levels and free radical generation have also been implicated in pathogenesis of vincristine induced painful neuropathy.[117-119] In the present study, vincristine treatment was noted to increase ca2+ levels as well as oxidative stress(indicated by elevated TBARS and decreased GSH level), supporting the contention that ca^{2+} and free radicals play a key role in development of vincristine induced neuropathy. Administration of IPC extract attenuated vincristine induced rise in calcium and TBARS levels and increases GSH levels. The results of this study proved that the antinociceptive and antineuropathic pain activity of IPC may be due to its anti oxidant and calcium channel inhibitory activity.

IPC has been reported for the presence of anti oxidant secondary metabolites like tannins and flavonoids. HPLC analysis of IPC revealed the presence of a flavonoid, kaempferol which has already been documented for antioxidant,

antinociceptive and anti-inflammatory activity. The present study also proved the anti inflammatory activity of IPC by *in vitro* membrane stabilization model. Hence anti neuropathic pain activity of IPC may be due to the presence kaempferol.

IN VITRO MEMBRANE STABILIZATION STUDY

The results obtained from the *in vitro* membrane stabilization study is presented in tab **.**

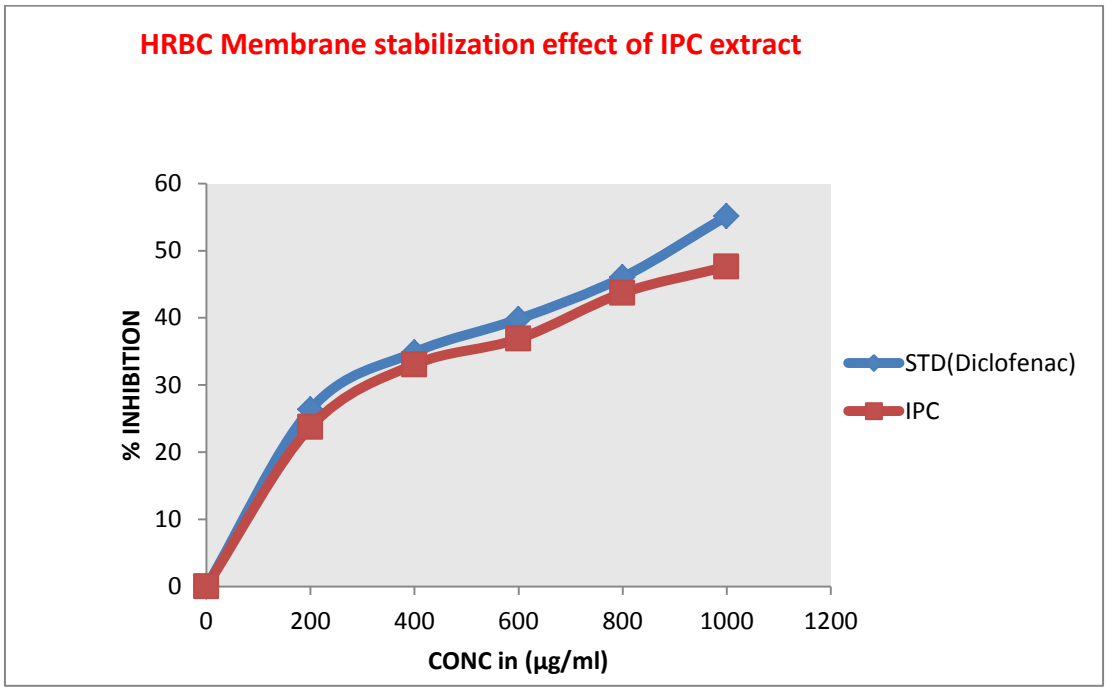
This study revealed that IPC extract showed significant membrane stabilization activity (**IC_{50} 944.8 µg/mL**) when compared with standard drug Diclofenac sodium(**IC_{50} 836.2 µg/mL**). The result was presented in **table 15**.**(fig 34)**

Table 15: Percentage of membrane stabilization by Diclofenac and IPC extract

Con.c in µg/ml	**Percentage inhibition of Diclofenc**	**Percentage inhibition of IPC extract**
200	26.3±0.30	23.7±1.90
400	34.8±0.99	33.0±0.44
600	39.8±0.11	36.8±0.56
800	46.0±0.71	43.7±0.26
1000	55.1±0.85	47.6±1.25
IC_{50}	**836.2µg/Ml**	**944.82µg/mL**

***mean of three readings ± SEM**

FIG 34: Percentage of membrane stabilization by IPC extract



Discussion [114-116]

Lysosomes are intracellular particles which contain most of the lytic and digestive enzymes of the tissue. The rupture of the lysosomes results in injury or death to surrounding tissues and also acute inflammation. The membranes of lysosomes and erythrocytes are destroyed by similar agents; hence a test was developed to measure the ability of compounds to stabilize erythrocyte membrane to heat hemolysis. RBCs when exposed to various injurious substances such as methyl salicylate, phenyl hydrazine, and hypotonic medium or over heat will cause lysis of membrane accompanied by heamolysis and oxidation of hemoglobin. RBCs membranes are easily susceptible to free radical mediated lipid peroxidation by break down of biomolecules. RBCs membranes are similar to lysosomal cells. All NSAIDs inhibited hemolysis while other type of compounds had no effect. Hence prevention of hypotonic and heat mediated RBCs membrane lysis taken as measure of anti inflammatory activity of drugs.

A study has reported that the flavonoids exert membrane stabilizing effect on lysosomes both *in-vitro* and *in vivo* in experimental animals. Kaempferol has already been reported for anti-inflammatory activity. In the present study, HPLC evaluation of IPC extract revealed the presence of kaempferol. The results of the present study also indicate that the anti-inflammatory activity of IPC extract may be due to the presence of kaempferol.[


CONCLUSIÓN

CHAPTER - VII

SUMMARY AND CONCLUSION

Many unknown and lesser know plants are used in folk and tribal medical practice as a source of medicine. The medicinal values of these plants are not brought in to the lime light of scientific world. One such plant is *Ipomoea pes-caprae.* Keeping this in view an attempt was made to bring the lime light of the commonly occurring plant *Ipomoea pes-caprae.*

This dissertation work covers an extensive study on the leaves of *Ipomoea pes-caprae* also known as beach morning glory belongs to the family convolvulaceae.

The pharmacognostical, phytochemical profile including preliminary phytochemical screening, analysis and quantification of kaempferol in the plant extract by HPLC and pharmacological studies were carried out for the *in vitro* anti-inflammatory activity and *in vivo* anti neuropathic pain activity in vincristine induced neuropathy model.

Pharmacognostical studies establishes macroscopical, microscopical and analytical standard and characterization of leaves of this plant. These evaluation can be used further as identification and standardization parameters of the leaves.

Preliminary phytochemical screening on the leaves of *Ipomoea pes-caprae* confirms the presence of tannins, flavonoids, sterols, carbohydrates and saponins. The quantification of total phenolic, flavonoid and tannin was determined for ethanolic extract of leaves of *Ipomoea pes-caprae* which were found to be **22.27 mg/g, 217.1 mg/g and 157.259 mg/g** respectively. It proves the significant concentration of these phytoconstituents in the ethanolic extract of *Ipomoea pes-caprae*. HPLC studies of IPC extract and standard kaempferol revealed that the presence of kaempferol in this extract and the t_R of standard and sample were found to be **5.87 mins** and **5.88 mins**

respectively. Kaempferol content present in this extract was quantified as **0.034 %**. So for kaempferol has not been reported from the leaves of this plant. This is the first time we have reported the presence of this compound from the leaves of this plant.

IPC showed significant radical scavenging activity for Hydrogen peroxide, DPPH, Total antioxidant and reducing power assay. This may be due to the presence of flavonoid constituent like **kaempferol.**

IPC extract exhibited significant anti inflammatory activity in the *in vitro* membrane stabilization model (**IC_{50} 944.8 µg/mL**) when compared with standard drug Diclofenac sodium (**IC_{50} 836.2 µg/mL**).

Flavonoid has been reported for *invitro* anti-inflammatory activity. Kaempferol also has already been reported for anti-inflammatory activity. Hence the anti-inflammatory activity of IPC extract may be attributed due to the presence of kaempferol.

IPC extract exhibited anti neuropathic pain activity against vincristine induced neuropathy model at the dose level of 200 mg and 300 mg/kg. This extract showed significant anti neuropathic pain activity by attenuating vincristine induced behavioral, biochemical and histopathological alterations in a dose dependent manner. This study also showed that antioxidant and calcium inhibitory potential was attributed for the anti neuropathic pain activity of IPC extract. Flavonoid phytoconstituents like kaempferol has been reported for anti oxidative, anti inflammatory, calcium inhibitory and antinociptive activity. Hence it was concluded that anti neuropathic pain activity of IPC extract may be due to the presence of **kaempferol**.

Future scope of the study

Further studies are needed to isolate the active principles from IPC extract and the same may be evaluated for anti neuropathic pain and anti inflammatory activity using various animal models. The isolated active principles as well as the extract may be developed in a suitable formulation and they may be marketed after subjecting them for appropriate clinical trial studies.

This formulations prepared from this drug will be beneficial to the patients those who are suffering from neuropathy of various etiologies such as drug induced neuropathy, alcoholic neuropathy and diabetic neuropathy. The currently available anti neuropathic pain drugs such as pregabalin, gabapentin amitriptylline, duoloxetine, and sodium valproate produces so many adverse effects and they are also not adequate to treat neuropathy.


References

CHAPTER - VIII

REFERENCES

1. Varier PS. Indian medicinal plants. 1st Edn, Vol 3, Orient Longmn Ltd, Madras. 1996;45-50.
2. http://www.herbal medicine.org.
3. Fabio Firenzuoli, Luigi Gori . **"Herbal medicine today,Clinical and research issues."** 2007; 4: 37-40.
4. Stedmen's Medical Dictionary 26th edition. 124.
5. Petter N, Bennett J Brown. **"clinical pharmacology"** 10th Edn 295,307.
6. Muthuraman A, Jaggi A, singh N, singh D. "Ameliorative effects of amiloride and pralidoxime in chronic constriction injury and vincristine induced painful neuropathy in rats." **European journal of pharmacology** 2008; 587; 104-111.
7. Ross and Wilson. **"Anatomy and physiology in health and illness."** 9th Edn, 188.
8. Gollwallia Medicine for students 22nd Edn, 584- 586.
9. Singh A, jaggi S, singh N, Vivek J. **"Animal Models in Neuropathic pain."** Fundamental and Clinical Pharmacology 2009:1,2,18.
10. Agrawal SS, Paridhavi M . **Textbook of Herbal Technology.** 1st Edn, University Press Private Ltd. Hyderabad. 2007; 219: 625.
11. Mukherjee PK. Quality control of herbal drugs: An approach to evaluation of botanicals. 1stEdn. **Business Horizons, New Delhi.** 2002:132-133, 161, 173, 186.
12. http//Herbal inflammation cures/eHow.org
13. Studio botanica.com/ 15- top- anti- inflammatory- herbs- species.
14. www.ethanomedicinal.org.ipc

15. Pareda-Miranda R , Escalanate-Sanchez E, Escobeda-Martinez C. Characterization of lipophilic pentasaccharides from beach morning glory *ipomoea pes-caprae*. **J Natural Products** 2005; 56(12): 1724-1726.

16. Esobedo-martinez C, Pereda-Miranda .Isolation of resin glycosides from *ipomoea pes-caprae.* **Journal of Natural Products** 2008; 70 (6): 974-978.

17. Deepak Venkataraman N, Clement Atlee W, Purushoth Prabhu T. Phyto Chemical analysis from *ipomoea pes-caprae.* **International Research Journals of Pharmaceutical and Applied Sciences** 2003; 3(4): 79-83.

18. Pongprayoon U. Pharmacologic studies on the Thai medicinal plant *Ipomoea pes-caprae* . **Actapharmaceutica Nordica** 1991; 3(3): 186.

19. Philippi AF, Joseph A, Skaria BP. Immunostimulatory activity of *Mutayaba elaeignoids* and I. *pes-capare* . **Journal of pharmacology** 2010; 28(6): 457-460.

20. Deepak Venkataraman N, Clement Atlee W, Purushoth PrabhuT. Antiiflammatory activity from *ipomoea pes-caprae.* **Journal of pharmacology** 2010: 4(9): 34-41.

21. Umamaheshwari G, Ramanathan T, Shanmuga priya R. Anti oxidant and radical scavenging activity of *Ipomoea pes-capare*. **International journal of Pharm tech research ;** 4(2): 848-851.

22. Deepak Venkataraman N, Clement Atlee W, Purushoth Prabhu T. Antiulcer and pylorus ligation effect from *ipomoea pes-caprae.* **Journals of Pharmaceutical and Applied Sciences** 2003; 3(4): 79-83.

23. Ramanathan T, Shanmugapriya R, Thirunavukkarasu P, Renugadevi G. *Invitro* antifungal activity from flower extracts of *Ipomoea pes-caprae.* **International Journal of Advances in Pharmaceutical Research** 2012;3(1): 732-735.

24. Martins FM, Lima JE, Mascanehas AAS, Macedo TP. Secretory structure of *I.asarifolia* anatomy and histochemistry. **Brazillan Journal of Pharmacognosy** 2012; 22(1): 13-20.

25. Rajendran K, Srinivasa KK, Shirwaikar A. Pharmacognostical identification of stem and root of *I.qumoclit(linn)* .**Natural products sciences** 2008; 13(4): 273-278.

26. Siddiqui TO ,Ahmad J, Sharma N. Botanical character of kaladana an important unani drug. **Hamdrad Medicus** 1990; 33(1):84-85.

27. Ono M, Fukuda H, Murata H, Miyahara K. Resin glycosides from *Ipomoea digitata.* **Journal of Natural Medicines** 2010;63(2): 176-180.

28. Nair GG, Methews A. Chemical and nutrient analysis from *Ipomoea carnea*. **Journal of Economic and Taxonomic botany** 2010;33(4):960-965.

29. Yin YQ, Huang XF , Kong LY, Niwa M. Isolation of Pentasaccharide resin from root of *Ipomoea batatas*. **Chemical and pharmaceutical bulletin** 2009;56 (12): 1670-1674.

30. Yahara S, Yoshimoto M, Yamakawa O. Phenolic compounds from the leaves of *Ipomoea batatas*. **Natl Med** 2002;56(3): 121.

31. Singh V, Gupta PC. A non ionic seed gum from *Ipomoea pes-tigridies.* **International Journal of Pharmacognosy.** 1995;3(3): 238-241.

32. Ono M, Kawasaki T, Miyahara K. Resin glycosides XI operculeins III, IV, IX, XVI, XVII and XVIII from *Ipomoea operculata.* **Chemical and Pharmaceutical Bull**. 1991; 39(10): 2534-2539.

33. Wilkison RE, Hardcastle WS, Mecormic KCS. Seed ergot alkaloid content of *Ipomoea coccinea, ipomoeaquamoclit, Ipomoea wrighti.* **J sci Food Agric** 1987 ;39(4):335-339.

34. Harison DA, Kulshreshtha DK. Chemical examination of *ipomoea dichora.* **Fitoterapia** 1986;57(1):29-32.

35. Schneides JA, Lee J, Naya Y, Nakanishi K, Oba K, Uritani I. The fate of the phytoalexin *Ipomoea marone*. **Phytochem.** 1984;23(94): 759-764.

36. Hwa KS , Chung DM, Chung YC, Chun HK .Hypouricemic effect from *Ipomoea batatas*. **Phytotherapy research.** 2012; 25(9): 1415-1417.

37. Leon Rivera I, Herrera Ruiz M, Estrada Soto S, Del Carmen Gutierrez M, et al. Cyto toxic effect of convolvulin from *Ipomoea tyrianthina*. **Journal of Ethanopharmacology** 2011;135(2): 434-439.

38. Chimkoden R, Patil MB, Jalapure SS ,Agarwal N,Tripathi R,Tripathi A, Patil NR. Wound healing activity from *Ipomoea batatas*. **Advances in pharmacology and Toxicology** 2010;10(3): 69-72.

39. Lin RJ, Chen CY. Cytotoxic activity from *Ipomoea cairica* .**Natural Products research.** 2009; 22(9): 747-753.

40. Herrera M, Gutierrez C, Jimenez Femer JE, Tortoriello J, Miron G, Leon I. Antidepresent activity from *Ipomoea stans*. **Journal of Ethanopharmacology.** 2008;112(2):243-247.

41. Datta choudhurry M, Chudurry DE, Paul SB. Antifungal and CNS depreesant activity of *I. aquatica.* **Bio science, Bio technology research Asia.** 2007; 4(1): 145-148.

42. Ferreria AA, Amarlal FA, Durate DG, Oliveria PM, Alves RB, Silveria D, Azevedo AO,Raslan DS,Castro MA. Antinociceptive effect from *Ipomoea ca*irica extract**. Journal of ethanopharmacology.** 2006;105(1-2):148-153.

43. Leon I, Enriquez RG, Nieto DA, Alonso D, Resholds WF, Aranda E, Villa J. Isolation of pentasaccharide glycosides from roots of *Ipomoea murucoides*. **Journal of Natural Products.** 2005;68(8):1141-1146

44. Yoshimot M, Kurataa RK, Fuji M, Hou DX, Ikeda K, Dome TY, Osako M. Phenolic composition and radical scavenging activity of sweet potato (*Ipomoea batatas)*derived schochu distillery by products treated Koji. **Bio sci Bio tech Bio chem.** 2004;68(12): 2477-2483.

45. Huueza IM, Fonsca ESM, Paulino CA, Haraguchi M, Gorniak SL. Evaluation of immunomodultory activity of *Ipomoea carnea* on peritoneal cells of rats. **Journal of ethanopharmacology**. 2003;87:181-186.

46. Mutsui T, Kobayashi EM, Sugita F, Terahara N, Matsumoto. Anti-hyperglycemic effect of discylated anthocyanin derived from *Ipomoea batatas* can be achieved through the alpha glucosidasae inhibitory action. **J Agric Food Chem** 2002; 50(25):7244-7248.

47. Kusano S, Abe H, Tamura H. Isolation of antidiabetic activity of *I.batatas*. **Bio sciences Bio technology and Bio chemistry** 2001; 65(1): 109-114.

48. Navarro-Ruiz A, Delamora GP, Villanuev Michel MI, Dominguez JR, Bastidas-Ramirez BE, Quezada-Arellano JD, Rui Madrigal B. Anticonvulsant effect of aqueous,hydro alcohol and choloroform extracts from *Ipomoea stans* root. **Phytotheraphy Res** 1996;10 (3):242-244.

49. Reynols WF, Enriquez RG, Gonzaler H, Leon I, Magos G, Villareal ML. Isolation and characterization of cytotoxic and anti bacterial tetrasaccharides, glycosides from *Ipomoea stans*. **J Natl Products** 1995;58(11):1730-1734.

50. Reza MS, Khan MOF, Islam MA, Chowdhury AKA. *In vitro* antimicrobial activity of *Ipomoea fistula*. **Fitoterapia** 1994;65(5):465-466.

51. Da SilvoFilho AA, Correa Lima RMO, De Andrade Chiapett A, DoNascimento SC, De Souza IA, Demello JF, Veith HJ. Anticancer and antimicrobial glycosides from *Ipomoea bahiensis.* **Phyto chem.** 1986; 25(5):1077-1081.

52. http://lee.ifas.ulf.edu/horticulturenew.htm http://

53. www.wikipidia.ipc.htm

54. Anish M, Ganesh N, Kharya M D. A new thirst in search of de-novo therapeutic approach . **"International Journal of Phytomedicine."** 2010;2 ; P- 18-21

55. Wallis. **Analytical Microscopy.** 3rd Edn, J & A Churchill Ltd, London;1965;180-184

56. Easu K, **"Plant anatomy".** John-wiley and sons, New York; 1964;767

57. .Easu K **"Anatomy of seed plants. "** Johnwiley and sons. New York;1979;550

58. Gamble JS. Flora of the presidency of Madras. Vol II & III. Botanical survey of india,Calcutta,India

59. Hendry AN , Kumari GR, Chitra V . Flora of Tamil Nadu, India, Botanical survey of India, Southern Circle, Coimbatore. Vol.3;258

60. Johansen DA. Plant micro technique. McGraw Hill Book Co. New York.1940;523

61. .Mathew KM . The flora of TamilNadu Karnaticvol. Polypetalae the Ranipetherbarium, St.John's college, Tiruchirapalli, India.1983;688, 689-1540.

62. Metcalfe C.R,Chalk L **"Anatomy of the dicotylendons"** Calerendonpress,Oxford. vol.I& II;1950.

63. Metcalfe C.R,Chalk L **"Anatomy of the dicotylendons"** Calerendon press,Oxford.vol.I;1979;276.

64. O'Brien TP, Feder N, Mcculs ME. Polychromatic staining of plnat cell walls by toluidine blue-O, Protoplasma. 1964;59:364-373.

65. Sass JE **"Elements of botanicals Micro technique."** McGraw Hill Book Co. NewYork ; 222.

66. Wallis TE **"Text book of Pharmacognosy"** CBS publishers and distributors, Shahdara, Delhi, India. 1985.

67. Yoganarasimhan SN. **"Medicinal plants of India**, TamilNadu, and Regional research institute (Ay), Bangalore, India. 2000; 715.

68. Kokate CK. **Practical Pharmacognosy** 4th edition, valla prakashan, New Delhi. 10-17.

69. Hari narayna SC, Pathak AK, Mukul T. Standardization of some herbal anti diabetic drug in poly herbal formulation. **Pharmacog research** 2011, 3; 49-56.

70. **The ayurvedic pharmacopoeia of india,** 1st edition, Govt of india. 2(1); 98-101, 128-130.

71. Trease and Evans. **Textbook of Pharmacognosy.** 5thedn. Elsevier Publishers, New Delhi. 74.

72. Kay LA. **Microscopical studies of Drugs**. 1stedn. Bailliere, Tindal and Cox, London, 1938;17-18.

73. Govt. of India, **Ministry of Health and Family Welfare Indian Pharmacopoeia,** controller of publications, New Delhi.1996; A53-A5

74. Bently and drivers. **The text book of Pharmaceutical chemistry,** 8th Edn, Oxford university press, London. 31.

75. Johansen DA. **Plant micro technique**. 1st Edn, McGraw Hill Book co, New York 1940 ; 222.

76. Shodhgangainflibnet ac. In 8080/ jspui/ bitstream/., 107_ chapter/ 282 pdf..

77. Quality control methods for Medicinal plant material. Geneva. 1998; 10-31.

78. Chase CR, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. **J Amer Pharm Assoc Sci Edn** 1949; 28:324-331.

79. Deepa VS, Kumar PS, Latha R. Antioxidant studies on the ethanolic extract of *commiphora* spp. **Afric J Biotech** 2009; 8; 1630-1661.

80. Harborne JB. Phytochemical methods. **A guide to modern techniques of plant analysis** 2nd edn, Chapman and Hall, London. 1994; 1-35.

81. Shah and Seth. **Textbook of Pharmacognosy and Phytochemistry**.1st edition 2010.Elsevier India Private Ltd. 234.

82. Kumazawa S, Taniguchi M, Suzuki Y, Shimura M, Kwon MS, Nakayam T. Antioxidant activity of polyphenols in carob pods. **J Agric Food Chem** 2002; 50: 373-77.

83. Singleton Vernon L, Rudolf O, Lamuela – Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by mean of FolinCiocalteu reagent.1979; 152.

84. Siddique MA, Mujeeb M, Najim AK, AkramM. Evaluation of antioxidant activity, quantitative estimation of phenols and flavanoids in different parts of *Aeglemarmelos*. **African J Plant Sci** 2010; 4(1):1-5

85. Estimation of total flavonoid content in *Propolis* by two complementary colorimetric methods. **J Food Drug Analysis** 2000; 10(3):178-82.

86. Mabry TJ, Markham KR, Thomas MB. The systematic identification of flavanoids. Springer Verlay, New York USA. 1970.

87. Olennikov DN, Tankhaeva LM. A method for quantitative estimation of total flavonoids in greater plantain leaves. **Pharmaceutical Chemistry Journal** 2008; 42(4):17-25.

88. Schanderal SH, **Method in food Analysis .** Academic press, New York 1970: 709

89. Jain UK, Dixit VK. Spectrophotometric estimation of tannins from the chyavan prash. **Indian drugs** 2004; 41: 469-472103.

90. Arit M, Naman K, Fareeds. Pharmacognostic studies and evaluation of phenolic contents of trunk bark of *Spondias mangifera willd.* **Natural Pro Red** 2009 ;(8); 146-150.

91. www.chemguide.co.uk/analysis/chromatography/hplc. html

92. Peter B, Ojong1, Victor Njiti, ZibaoGuo, Ming Gao, Samuel Besong Sandra L, Barnes. Variation of Flavonoid Content AmongSweet potato Accessions. 133(6): P-819-824.

93. Nagendran B, Ravigadevi S, KalyanaSundram J , Samir Samman Y. Antioxidant properties of palm fruit extracts. **Asia Pac J Clin Nutr** 2005; 4(4):319-324.

94. Rajan S, MahalakshmI S, Deepa VM, Sathy K, Shajitha S, Thirunalasundari T. Antioxidant potentials of *Punicagranatum* fruit rind extracts. **International Journal of Pharmacy and Pharmaceutical Sciences** 2011; 3(3):45-47.

95. Ya-Li Wang, Xiao-Dong Wang , Bing Zhao , Yu-Chun. Enhancing antioxidative capacity of *Lepidium meyenii* by *addition* of methyl salicylate to culture medium .**Wang Acta Physiol Plant** 2007; 29:417–423

96. Da Porto C, Calligaris S, Celotti E, Nicoli MC. Antiradical properties of commercial congas assessed by the DPPH test. **J Agri Food Chem** 2000; 48: 4241-4245

97. Jananie RK, Priya V , Vijaya lakshmi K. *Invitro* assessment of free radical scavenging activity of *Cynodon dactylon.* **J Chem Pharm Res** 2011;3(4): 647-654.

98. Jan Muselik , Maria Garcia-Alonso , Maria Martin-Lopez P, Milan Zemlicka, Julian Rivas-Gonzalo C. Measurement of Antioxidant Activity of Wine Catechins, Procyanidins, Anthocyanins and Pyranoanthocyanins. **Int J Mol Sci** 2007; 8(7): 797-809

99. Prieto P, Pineda M and Aguilar M. Spectroscopic quantitation of antioxidant capacity through the formation of a Phosphomolybdenum complex specific application to the determination of vitamin of E. **Anal Biochem** 1999; 269: 337-341.

100. Nilgun, MuzafferTunce, I smuhanPotoglu-Erkara. Phenolic compounds and antioxidant activities of some Hypericum species. A comparative study with *H. perforatum*. **Pharmaceutical Biology** 2009; 47(2): 120–127

101. Benzie FF and strain JJ. Ferric Reducing Antioxidant Power Assay, Direct Measure of Total Antioxidant activity of Biological Fluids and Modified Version for simultaneous Measurement of Total Antioxidant Power and Ascorbic acid concentration. **Methods on Enzymology** 1999; 299: 15-23.

102. Raghu KL, Ramesh CK, Srinivasa TR and Jamuna KS. Total antioxidant capacity in aqueous extract of some common vegetables. **Asian J Exp Bio Sci** 2011; 2(1): 58-62.

103. Eddy NB, Touch berry CF, Lieberman JE. Synthetic analgesics: I. Methadone isomers and derivatives. **J Pharmacol ExpTher** 1950; 98: 121-137.

104. Chaplan SR, Bch FW, Posrel JW, Chung JM, Yaksh JL. Quantitative assessment of tactile allodynia in the rat paw. **J Neurosci Methods**.1994; 53: 55-63.

105. Necker R, Hellen RF. Noxious thermal input from the rat tail, Modulation by descending inhibitory influences Pain.1978 4: 231-242.

106. Okhawa H, Ohesil N, Yasik. Assay of lipid Peroxidation in animal tissue by TBARS, **Analyst Biochem** 1979; 95: 351-358.

107. Ellman GL. Tissue sulfhydryl groups. **Arch Bio chem Bio phys** 1959; 82: 70-77.

108. Severinghaus JW, Feru bee JW. Calcium determination by flame photometry: Methods of serum, urine and other fluids. **J Biol Chem** 1950; 187: 621-630.

109. Muthuraman A, Diwan V, Jaggi AS, Singh N, Singh D. Ameliorative effects of *Ocimum santum* in sciatic nerve transaction-induced neuropathy in rats. **J Ethanopharmacol** 2008; 120: 56-62.

110. Sodoh T, Desai SP, Haderer AE, Sudoh S, Gerner P, Anthony DL, De Girolami U, Wang GK. Neurologic and histopathologic evaluation after high volume intrathecal amitriptyline. **Anaesth. Pain Med** 2004; 29: 434-440.

111. Sadique J, Al- Rqobah WA, Bhugharith MF, El-Gindi AR. The bioactivity of certain medicinal plant on the stabilization of RBC membrane system. **Fitoterpia** 1989; 60: 525-532.

112. Das Feirrali M, Signormi C, Ciccoli L, Comporti M. Iron release and membrane damage in erythrocytes exposed to oxidaising agents, phenyl hydrazine, devicenne and isouranil. **Biochem J** 1992; 285 : 295-301.

113. Coyne WE. Non steroidal anti inflammatory agents and antipyretic in medicinal chemistry 3rd edn Alfred Burgrer Ed. **Wiley Inter science** New York. 953- 975.

114. Middleton JE, Biological activity properties of plant flavonoids, an overview. **Int J Pharmacognosy** 1996 ; 34: 344-348.

115. Oyedapo OO, Famurrew AJ. Antiprotease and membrane stabilizing activities of extracts of Fagarazanthoxyloides, Olaxsubscorpioides and tetra pleura tetraptera. **Int J Pharmacogn** 1995; 33: 65-69.

116. Oyedapo OO. Red blood cell membrane stabilizing potentials of extracts of Lantana camera & its fraction inter. **J of plant Physiology and Biochemistry** 2010; 2(4): 46-51.

117. Kamei J, Tamura N, Saitoh A. Possible involvement of the spinal nitric oxide/cGMP Pathway in vincristine induced painful neuropathy in mice. 2005; 117:112-120.

118. Siau C, Bennett GJ. Dysregulation of neuronal calcium homeostasis in chemotherapy- evoked painful peripheral neuropathy. **Anesth, Analg** 2006; 102:1485-1490.

119. Sudoh Y, Desai SP, Haderer AE, Sudoh S, Gerner P, Anthony DC, Wang GK. Neurological and histopathologic evaluation after high volume intratheal amitriphyline. **Reg Anesth pain Med** 2004; 29: 434-440.

ANNEXURE

Investigator declaration

- I certify that I have determined that the research proposal herein is not unnecessarily duplicate of previously reported research.
- I certify that all individuals working on this proposal and experimenting on the animals have been trained in animal handling procedures.
- For procedures listed under item 11, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.
- I will obtain approval from the IAEC / CPCSEA before initiating any significant changes in this study.
- Certified that performance of experiment will be initiated only up on review and approval of scientific intent by appropriate expert body (institutional scientific advisory committee / funding agency / other body (to be named)
- Institutional biosafety committee (IBC) certification of review and concurrence will be taken (required for studies utilizing DNA agents of human pathogens)
- I shall maintain all the records as per format (Form D)

P.Anitha.

Signature

(P.Anitha)

Name of Investigator

I. A. E. C. CHAIRMAN
INSTITUTIONAL ANIMAL ETHICAL COMMITTEE
K. M. COLLEGE OF PHARMACY
MADURAI-625 107.